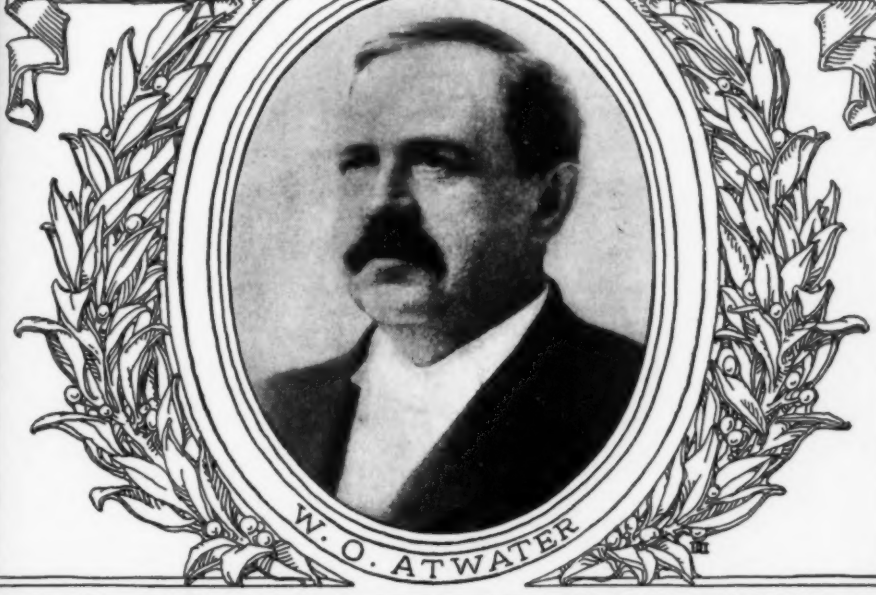
OCTOBER 11, 1943

THE JOURNAL OF NUTRITION

VOLUME 26 NUMBER 4



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Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA 4, PA.
Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign

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WOODLAND AVENUE AND THIRTY-SIXTH STREET, PHILADELPHIA 4, PA.

FIELD PEAS AS A SOURCE OF PROTEIN FOR GROWTH [1]

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(Received for publication February 15, 1943)

Finks, Jones and Johns ('22) reported that the field pea (Pisum sativum) when fed as 75% of the diet had given "normal growth" in young rats, and the opinion has been very generally held that the protein of the field pea supplies adequate amounts of all the nutritionally essential amino acids. Deficiencies which had occurred in other experiments could always be explained as due to the use of other legumes designated by the common name, pea. Thus Finks and his co-workers had found that cystine was a limiting factor in growth with the cowpea (Vigna sinensis), and McCollum, Simmonds and Parsons ('19) had reported that split peas identified as Vicia sativa, when soaked, autoclaved and dried in air, did not support very good growth if fed as 45% of the diet. Sure ('21) reported that in repeating McCollum's work he encountered conflicting results which could be traced to the inadvertent use of one lot of field peas. More recently Cook and Robertson ('41) have reported that the protein of Alaska field peas when added to a cereal ration for chicks has about 50% of the supplementary value of casein and that dl-valine does not appear to be the limiting factor in the nutritive value of pea protein.

Finks and his co-workers ('22) described their product as a smooth, yellow, split pea. The Alaska field pea, also identified

[1] Published with the approval of the Director as research paper no. 213 of the Idaho Agricultural Experiment Station.

THE JOURNAL OF NUTRITION, VOL. 26, NO. 4
OCTOBER, 1943
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as Pisum sativum, is a smooth, round pea green in color. In the immature state these peas are used chiefly for canning purposes and when mature are marketed as split peas for human consumption. Dried peas are becoming increasingly valuable as a source of protein for low cost diets. Field peas and their by-products are used extensively in animal feeding. This report deals with the nutritive value of peas, both raw and heat-treated, as the sole source of protein and the effect on growth in young rats by the addition of dl-methionine and of l-cystine to the diet.

EXPERIMENTAL METHOD

Alaska field peas (Pisum sativum), grade no. 1,[2] were cleaned, culled, and ground in a Wiley mill through a 1 mm. sieve. After grinding, the peas were well mixed and divided into three portions. For heat treatment one portion was spread out in layers approximately ½ inch thick and baked in a Freas oven for 1½ hours at 140° C., and another portion was spread out about 1 inch thick and autoclaved for 1½ hours at 17 pounds pressure after which they were dried at room temperature. The third portion was fed in the raw state.

Raw, baked and autoclaved peas were analyzed for moisture and for nitrogen, and the quantity of each required to furnish a protein level (N × 6.25) of 10% was used in making up the diets. A diet containing 10% vitamin-free-casein was used as a standard of comparison in the feeding tests. The composition of the diets is presented in table 1.

The added amino acids, dl-methionine and l-cystine, when used were thoroughly mixed with the diets, replacing an equivalent amount of sugar without adjustment in the nitrogen of the diet.

Each rat received as a supplement six times a week pure vitamins[3] as follows: thiamine hydrochloride 20 µg., ribo-

[2] Graded peas were furnished by the Department of Agronomy of the University of Idaho.

[3] Generous amounts of thiamine, riboflavin, pyridoxine and calcium pantothenate were furnished by Merck and Co., Inc.

flavin 20 μg., pyridoxine 20 μg., calcium pantothenate 80 μg., nicotinic acid 250 μg., choline hydrochloride 10 mg.

The rats were 21 or 22 days old when started on the test diets. They were housed in individual cages and weighed weekly for 8 weeks. Food consumption records were made at each weighing.

The statistical analyses [4] of the data were made according to the methods of Snedecor ('40).

TABLE 1

Composition of diets used in pea protein studies.

INGREDIENT	CASEIN		PROTEIN IN PEAS			
			Raw		Baked	Autoclaved
	10%	18%	10%	20%	10%	10%
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Raw peas	...	...	438	876	...	...
Baked peas	...	...	...	...	405	...
Autoclaved peas	...	...	...	...	...	463
Casein [1]	100	180	...	...	...	...
Salt mixture [2]	40	40	40	40	40	40
Cottonseed oil [3]	30	30	30	30	30	30
Cod liver oil	20	20	20	20	20	20
Cane sugar	790	690	472	34	505	447
Agar	20	40	...	...	...	...
	1,000	1,000	1,000	1,000	1,000	1,000

[1] Labco.

[2] Osborne and Mendel, 1919, J. Biol. Chem., vol. 37, p. 572.

[3] Wesson.

RESULTS AND DISCUSSION

The results of this series of experiments are summarized in tables 2 and 3. It is evident that the lack of available methionine is the principal growth-limiting deficiency of raw peas (lots 3, 4, 5, 6). The rates of gain were approximately four times greater in a period of 8 weeks when raw peas were supplemented with 0.3 to 0.9% methionine. Furthermore, rats fed on peas plus 0.3% methionine gained 47% more in 8 weeks than those receiving casein at the same protein level, and re-

[4] Credit is due M. L. Buchanan, Department of Animal Husbandry, for valuable assistance in statistical treatment of data.

TABLE 2

Average growth response by rats fed diets used in the investigation of pea protein.

LOT NO.	DIET	NUMBER OF RATS	AVERAGE GAIN IN 8 WEEKS	AVERAGE GAIN PER GRAM OF PROTEIN	AVERAGE FOOD REQUIRED PER GRAM OF GAIN	AVERAGE FOOD CONSUMED DAILY PER RAT
			gm.	*gm.*		
1	10% casein	21	85.2 ± 7.2 [1]	1.74 ± 0.03 [1]	5.65 ± 0.12 [1]	8.49 ± 0.62 [1]
2	18% casein	3	151.3 ± 0.4	1.66 ± 0.45	3.36 ± 0.05	9.07 ± 0.14
3	10% raw pea protein	22	29.2 ± 1.6	1.05 ± 0.05	9.96 ± 0.54	4.89 ± 0.10
4	10% raw pea protein plus 0.3% methionine	7	124.7 ± 7.3	2.35 ± 0.05	4.22 ± 0.12	9.34 ± 0.42
5	10% raw pea protein plus 0.6% methionine	12	123.4 ± 5.8	2.58 ± 0.04	4.24 ± 0.49	8.52 ± 0.35
6	10% raw pea protein plus 0.9%	5	107.2 ± 11.9	2.36 ± 0.10	4.28 ± 0.20	8.02 ± 0.57
7	10% raw pea protein plus 0.5% cystine	7	35.4 ± 2.0	1.35 ± 0.05	7.50 ± 0.27	4.69 ± 0.12
8	20% raw pea protein	3	86.3 ± 3.5	1.00 ± 0.06	5.01 ± 0.33	7.70 ± 0.41
9	10% baked pea protein	10	20.1 ± 2.0	0.78 ± 0.05	13.30 ± 1.08	4.48 ± 0.17
10	10% autoclaved pea protein	15	15.1 ± 1.0	0.59 ± 0.03	18.10 ± 1.25	4.59 ± 0.12
11	10% autoclaved pea protein plus 0.6% methionine	6	118.1 ± 5.8	2.35 ± 0.05	4.27 ± 0.01	8.59 ± 0.24
12	10% autoclaved pea protein plus 0.5% cystine	3	30.0 ± 1.5	1.16 ± 0.01	8.20 ± 0.08	4.59 ± 0.17

[1] Standard error.

TABLE 3

Statistical comparisons of growth on various diets.

LOT NO.	DIETS COMPARED	GRAMS GAIN IN 8 WEEKS			GRAMS GAIN PER GRAM PROTEIN		
		Mean difference	Standard error of difference	t [1]	Mean difference	Standard error of difference	t [1]
1–3	10% casein vs. 10% raw pea protein	56.0	± 7.3	7.67	0.69	± 0.06	11.50
3–10	10% raw pea protein vs. 10% autoclaved pea protein	14.1	± 1.8	7.83	0.46	± 0.06	7.60
3–9	10% raw pea protein vs. 10% baked pea protein	9.1	± 2.5	3.64	0.27	± 0.07	3.80
3–4	10% raw pea protein vs. 10% raw pea protein plus 0.3% methionine	95.5	± 7.4	12.91	1.30	± 0.07	18.50
3–5	10% raw pea protein vs. 10% raw pea protein plus 0.6% methionine	94.2	± 6.0	15.70	1.53	± 0.06	25.50
3–6	10% raw pea protein vs. 10% raw pea protein plus 0.9% methionine	78.0	± 12.0	6.50	1.31	± 0.11	11.91
3–7	10% raw pea protein vs. 10% raw pea protein plus 0.5% cystine	6.2	± 2.5	2.48	0.30	± 0.07	4.20
10–11	10% autoclaved pea protein vs. 10% autoclaved pea protein plus 0.6% methionine	103.0	± 5.9	17.40	1.86	± 0.06	31.00
10–12	10% autoclaved pea protein vs. 10% autoclaved pea protein plus 0.5% cystine	14.9	± 1.5	9.93	0.57	± 0.03	19.00
2–8	18% casein vs. 20% raw pea protein	97.9	± 6.0	16.32	1.30	± 0.07	18.57
4–5	10% raw pea protein plus 0.3% methionine vs. 10% pea protein plus 0.6% methionine	1.3	± 9.3	0.14	0.23	± 0.06	3.83
5–6	10% raw pea protein plus 0.6% methionine vs. 10% pea protein plus 0.9% methionine	16.2	± 13.2	1.23	0.22	± 0.10	2.20

[1] A t value of 3.707 establishes the 1% point for the comparisons based on the smallest number of degrees of freedom; therefore a value of 3.707 or larger is highly significant.

quired 25% less food per gram of gain (lots 1, 4). Supplementing the raw pea diet with 0.3% or more methionine practically doubled the food consumed daily (lots 3, 4, 5, 6) and decreased the amount of food required per gram of gain by one-half.

The most efficient level of supplementary methionine for 10% raw pea protein appears to be not more than 0.3% of the diet. Levels of 0.6 and 0.9% caused no increase in rate of gain or in average gain per gram of protein over that with 0.3%.

The results given in table 2 show that the baking and autoclaving of field peas decreased its value for growth of rats when compared with raw peas. The rate of gain observed on autoclaved peas was one-half as much as on raw peas (lots 3, 10) and baking decreased the gains but not as greatly (lots 3, 9). Apparently some nutritional factor associated with protein adequacy is destroyed by both dry and moist heat. Field peas react differently to heat treatment than do soybeans, for Hayward, Steenbock and Bohstedt ('36) reported improvement in the nutritive value of soybean protein when subjected to heat treatment. According to Hayward and Hafner ('41), autoclaving soybeans possibly increases the availability of cystine and methionine as well as other essential amino acids. Morgan ('31), however, found that dry heating damaged cereal proteins and that the injurious effect could be corrected by feeding histidine or lysine with the heated protein. Autoclaving peas seems to destroy cystine instead of making it more available. The evidence for this conclusion is the fact that the addition of 0.5% cystine to the autoclaved pea diet gave a significant increase in rat growth (lots 10, 12, table 3), while supplementing raw peas with 0.5% cystine gave no significant increase in rate of growth (lots 3, 7, table 3). Since the growth rate on a diet of autoclaved peas and 0.5% cystine is the same as the growth obtained on raw peas, it is concluded that moist heat destroys cystine in peas. In a protein study with canned garden peas which had been cooked, Mitchell and Beadles ('30) showed that the addition of cystine to the diet significantly increased the rate of growth of rats.

The rats on the raw, baked and autoclaved pea diets ingested approximately the same amount of food daily, 4.89, 4.48, and 4.59 gm., respectively, while there was a marked decrease in the efficiency of food utilization by the rats fed heat treated peas. The average food required per gram of gain was, respectively, 9.96, 13.30 and 18.10 gm. These data clearly indicate that the differences obtained showing a decreasing effect of heat on the nutritive value of peas were not due to a lowered food intake. Instead, they suggest that either baking or autoclaving tends to destroy some factor, possibly cystine or methionine, which contributes to the more efficient utilization of the diet. The fact that adding 0.5% cystine to autoclaved peas made them equal in growth-promoting properties to raw peas and that the addition of methionine alone produced good growth substantiates the hypothesis that heat treatment, rather than altering the digestibility, destroys a specific nutritional factor, probably cystine.

The addition of 0.6% methionine to the autoclaved pea diet gave a growth response approximately equivalent to raw peas plus methionine, revealing that the loss due to autoclaving may be replaced with methionine. That cystine in animal tissues can be derived from methionine in the diet has been shown by Dawbarn ('38), Beach and White ('39), and Rose and Wood ('41) by measuring the increment of tissue cystine, especially hair, when methionine supplied the dietary sulfur, and by Tarver and Schmidt ('39) who recovered radioactive sulfur from cystine in rat tissue after feeding methionine containing S^{35}.

Mitchell ('38) explains the supplementing effect of cystine to a low methionine diet as due to the need of the animal for both amino acids, the cystine portion of which can be met by cystine itself or can be synthesized by the animal from methionine.

If the diets are examined on the basis of gain per gram of protein eaten, it is evident that raw pea protein at 1.05 compares more favorably with casein at 1.74 than it does when compared on actual gains. The addition of cystine (1.35) im-

proved the efficiency of raw peas somewhat, and methionine (2.35) raised it well beyond the casein value (1.74). Autoclaving the peas reduced the gain per gram of protein from 1.05 to 0.59 while supplementing the autoclaved peas with methionine increased the efficiency to 2.35 per gram of protein. On the same basis the cystine addition increased the efficiency of the autoclaved peas to 1.16, somewhat above the value for raw peas only, but not quite equal to raw peas plus cystine.

Rats fed a diet of raw peas at a 20% protein level made gains comparable to those receiving 10% casein but not what could be called normal with present-day standards, and the gain per gram of protein was essentially the same as for the 10% pea protein diet.

Notwithstanding the apparently good growth obtained from split peas at a 20% protein level by Finks, Jones and Johns, they report that for a 10-week period the gain per gram of protein eaten was only 1.01. This is approximately the efficiency of the Alaska pea for the 8-week test in this investigation (table 2), whether fed to give 10 or 20% protein diets.

In order to check the adequacy of choline, liver fat analyses for a few rats were determined. The fat values were as follows: On the 10% casein diet, 4.0% (average of six animals); raw pea diet, 2.8% (three animals); raw peas plus cystine, 2.1% (three animals); and raw peas with methionine, 2.8%. The animals on the stock diet used in this laboratory show around 2.3% fat in the liver at this age. These data indicate that the added methionine was not needed to supply labile methyl groups for the synthesis of choline.

SUMMARY

1. Raw and heated Alaska field peas (Pisum sativum) have been studied as the sole source of protein in diets for growing rats.

2. The principal growth-limiting deficiency in raw pea protein is methionine.

3. Baking and autoclaving field peas decreased the growth-promoting properties of the protein but did not change the food intake.

4. The addition of cystine to the autoclaved peas permitted a rate of growth comparable to that with raw peas.

5. Rats fed raw pea protein (10% level) plus 0.3 methionine as the sole source of protein made 47% more gain and required 25% less food per unit gain, than rats fed casein at a 10% protein level.

6. Apparently the Alaska field pea is an excellent source of the amino acids essential for growth, with the single exception of methionine.

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THE RATIO OF ASCORBIC ACID, RIBOFLAVIN AND THIAMINE IN RAW AND PASTEURIZED MILK[1]

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(Received for publication March 29, 1943)

Milk has long been recognized as possessing unique nutritive value. It contains protein of high biological value, minerals required for the growth and maintenance of the body, and many of the vitamins essential for human well-being. Because of the importance of the latter, many studies have been made of the vitamin content of cows' milk and the literature contains considerable data concerning the ascorbic acid, riboflavin and thiamine contents of milk produced in many localities under a variety of conditions. However, the extensive literature which was consulted did not contain a single assay of a milk for all three vitamins, ascorbic acid, riboflavin, and thiamine. Accordingly, it seemed desirable to determine the amount of these vitamins present, before and after controlled pasteurization of milk produced under standardized conditions.

EXPERIMENTAL

Source of milk

As a matter of convenience, winter milk (Jan., Feb., and March) was used for this study. It was produced by the college herd of sixty-seven cows which consisted of eighteen Ayrshires, thirteen Guernseys, eighteen Holsteins, eleven Jerseys, and seven Shorthorns. The animals were all normal, in a healthy condition, and under constant veterinary supervision.

[1] Contribution no. 478, Massachusetts Agricultural Experiment Station.

The stage of lactation, computed as of the middle of the experimental period, averaged 120 days for the Ayrshires, 134 days for the Guernseys, 133 days for the Holsteins, 142 days for the Jerseys, and 115 days for the Shorthorns, or for the herd as a whole, 129 days.

The daily ration of the cows consisted of 6 lbs. of locally grown mixed hay, 18 lbs. of corn silage, 15 lbs. of grass silage, 3½ lbs. of beet pulp, and 9 lbs. of grain containing 14.5% of protein. Because of war restrictions, the ration was lower than normal in concentrates. However, the cows produced an average of 15 quarts of 4% butterfat milk per day.

Pasteurization

The milk from the 3:30 P.M. milkings was promptly cooled and held at 40° F. until the following morning. After the milk obtained from the 3:30 A.M. milking had been cooled, the two were combined and taken to the pasteurizer. Hence, the combined milk was about 10 hours old when it was pasteurized. When the milk had been thoroughly mixed in the pasteurizer, a sample of "raw" milk was taken for analysis. Thus, the studies reported below were conducted upon the total day's production. The milk was pasteurized by the so-called holding process in an 800-quart stainless steel vat pasteurizer.

The time required for the preliminary heating of the milk varied from 27 to 98 and averaged 48 minutes. The temperature of pasteurization was 144° F. and the duration was from 30 to 37 (average 33) minutes.

As soon as pasteurization was completed, the milk was rapidly cooled to 40° F. A sample of pasteurized milk typical of the 800 quarts was taken for analysis and assays for riboflavin and ascorbic acid were commenced immediately but the thiamine assays were not started until 5 hours or so later.

Assay procedures

The ascorbic acid content of the milk was determined by the procedure reported by Holmes, Tripp, Woelffer and Satterfield

('39) and Tripp, Satterfield and Holmes ('37), which was adapted from the 2–6 dichlorophenolindophenol method of Bessey and King ('33).

The riboflavin assay procedure used in this study was a modification of methods suggested by Hand ('39), Hodson and Morris ('39), and Kemmerer ('41).

Twenty-five cubic centimeters of milk were added slowly to 50 cc. of acetone U.S.P. and filtered. A 35 cc. aliquot of the filtrate was diluted with distilled water, and stannous chloride and sodium hyposulphite were added to reduce all of the materials which produce fluorescence. The mixture was made up to 250 cc. and the riboflavin was re-oxidized by thorough agitation with air. Subsequently three 50 cc. aliquots were transferred to 125 cc. Erlenmeyer flasks. One cubic centimeter of standard riboflavin solution was added to aliquot "b" which served as a positive control. Aliquot "c" was reduced with 1 cc. of sodium hyposulphite solution and served as a negative control, which contained all substances, except riboflavin, present in aliquot "a" (the sample) and aliquot "b". The cubic centimeters of each aliquot were transferred to the cuvettes for fluorometric assay. The assays were made with a spectrophotometer [2] adapted for making fluorescence measurements. By reading the negative control "c" both before and after sample "a" and aliquot "b", it was possible to detect instantly any changes in the electric current, the setting of the instrument, or other mechanical factors which would affect the accuracy of the reading. Such constant checking of the instrument is not possible when one relies upon a reference curve for interpreting the reading of the sample.

The readings for "a", "b", and "c" supply data for computing the riboflavin content of the sample "a".

Since riboflavin may be destroyed by light (Hogan and Hunter, '28; Supplée, Ansbacher and Bender, '35; György, '35; and others) amber glassware was used and the assays were made in a specially prepared semi-basement laboratory

[2] Coleman Universal Spectrophotometer.

from which all daylight was excluded. The 100-watt Mazda bulbs were surrounded with long metal cylinders that prevented direct light reaching the laboratory tables at the side of the room. Meter measurements of the light intensity at the site of the precipitation, filtration and other assay procedures showed the light to be from one to two candle power.

The thiamine content of the milk was determined by a modification of the thiochrome method of Hennessy and Cerecedo ('39).

RESULTS

The ascorbic acid content of the raw milk (table 1) varied from 14.0 to 22.5 mg. per liter, with an average of 19.7 $\pm$ 1.8 mg. per liter. After the milk had been pasteurized its ascorbic acid content varied from 7.0 to 19.1 mg. per liter and averaged 15.9 $\pm$ 2.7 mg. per liter. The change in the ascorbic acid value of the milk from 19.7 mg. to 15.9 mg. per liter represents an 18.3% loss caused by pasteurization. This result is practically identical with the 18.71% loss reported in an earlier investigation by Holmes, Tripp, Woelffer and Satterfield ('39) in which herd milk from 175 Guernsey, Holstein, and Ayrshire cows was used. The 5 months' experimental period began in mid-August. The same assay procedure was followed, and the equipment was of the same type, namely, commercial size, stainless steel, spray vat equipped with stainless steel agitators that kept the milk gently in motion during the holding period which was for 30 minutes at 144° F. However, the potency of the milk was different for the two studies. In the earlier study, the values were 17.26 and 14.03 mg. per liter for the raw and pasteurized certified milk, whereas in the present study the values were 19.7 and 15.9 mg. of ascorbic acid per liter, respectively, for the raw and pasteurized milk. Thus, the results obtained in the two studies indicate very strongly that approximately one-fifth of the ascorbic acid content of fresh whole milk is lost during pasteurization in stainless steel equipment for 30 minutes at 143°–144° F.

Referring to table 1, it will be noted that the riboflavin content of the thirty-one samples of raw milk varied from 1.35 to

TABLE 1

The ascorbic acid, riboflavin and thiamine content of milk.

SAMPLE NO.	ASCORBIC ACID		RIBOFLAVIN		THIAMINE	
	Raw	Pasteurized	Raw	Pasteurized	Raw	Pasteurized
	mg. /liter		*mg. /liter*		*mg. /liter*	
1	21.0	15.0	1.41	1.42	0.33	0.31
2	20.5	15.7	1.52	1.51	0.35	0.29
3	19.6	14.9	1.42	1.42	...	...
4	19.0	14.5	1.56	1.54	0.33	0.29
5	19.6	15.0	1.47	1.37	0.35	0.31
6	17.6	12.6	1.53	1.48	0.32	0.32
7	21.5	17.7	1.71	1.67	0.32	0.32
8	21.5	17.7	1.56	1.52	0.33	0.29
9	22.5	8.8	1.52	1.42	0.32	0.26
10	20.1	15.1	1.55	1.52	0.33	0.32
11	20.0	15.0	1.56	1.50	0.34	0.32
12	21.4	17.4	1.75	1.67	0.33	0.30
13	14.0	7.0	1.58	1.49	0.32	0.30
14	18.7	14.1	1.39	1.47	0.30	0.28
15	20.8	18.0	1.58	1.69	0.32	0.30
16	18.2	14.6	1.50	1.49	0.35	0.33
17	19.2	16.3	1.56	1.48	0.35	0.32
18	20.5	17.0	1.49	1.40	...	0.21
19	20.2	17.1	1.35	1.45	0.33	0.31
20	20.1	16.6	1.44	1.19	0.34	0.30
21	22.1	19.1	1.49	1.34	0.33	0.31
22	19.9	18.7	1.43	1.43	0.34	0.32
23	16.0	18.1	1.55	1.48	0.34	0.32
24	18.5	18.5	1.72	2.06	0.34	0.24
25	20.9	19.0	...	...	0.34	0.31
26	19.2	15.3	1.42	1.34	0.31	0.29
27	20.7	17.7	1.38	1.45	0.29	0.29
28	19.6	17.2	1.44	1.33	0.31	0.30
29	20.3	17.2	1.48	1.46	0.35	0.32
30	20.1	17.4	1.54	1.42	...	0.34
31	20.6	18.2	1.48	1.36	0.32	0.31
32	17.0	13.6	1.52	1.60	0.33	0.32
Average	19.7	15.9	1.51	1.48	0.33	0.30
	± 1.8[1]	± 2.7	± 0.09	± 0.01	± 0.02	± 0.03

[1] Standard deviation.

1.75 mg. per liter with an average of 1.51 ± 0.09 mg. per liter. The amount of riboflavin in the pasteurized milk varied from a minimum of 1.19 mg. to a maximum of 2.06 mg. per liter, with an average of 1.48 ± 0.01 mg. per liter. Thus in these experiments the pasteurizing process caused a loss of approximately 2% of the riboflavin. This result is in very good agreement with those of Javillier ('40), who reported that preheating and sterilizing milk caused no loss of riboflavin, and those of Houston, Kon and Thompson ('40), who found the riboflavin content of February, June, and July milk to be slightly increased after both pasteurization and sterilization.

The thiamine content of the raw milk varied from 0.29 to 0.35 mg. per liter with an average value of 0.33 ± 0.02 mg. per liter. After pasteurization the thiamine content varied from a minimum of 0.21 mg. to a maximum of 0.34 mg. per liter and the average value for the thirty-one samples was 0.30 ± 0.03 mg. per liter of milk. Thus 9.1% of thiamine was lost during pasteurization of the milk by the holding process. This value is much less than that reported by a number of other investigators. Elvehjem ('41) reported that pasteurization caused a 20% loss of thiamine. Weckel ('36) found that commercial sterilization destroys approximately one-third of the vitamin B_1 in milk; and Houston, Kon and Thompson ('40) state that up to 50% of vitamin B_1 is destroyed in the course of heat treatment.

The ratio of ascorbic acid, riboflavin and thiamine in raw and pasteurized milk has been computed to show the relation of the riboflavin content to those of thiamine and of ascorbic acid, and the relation of ascorbic acid to thiamine.

The ratio of ascorbic acid to riboflavin in the raw milk varied from a minimum of 8.9 for sample 13 to a maximum of 15.0 for samples 19 and 27. Considering the group of samples as a unit, the average ascorbic acid content of the raw milk was 13.1 ± 1.43 times that of the riboflavin. The ratio of ascorbic acid to riboflavin in the pasteurized milk was somewhat lower than for the raw milk because of the loss of about one-fifth of the ascorbic acid during the pasteurization process. The mini-

mum value was 4.7 for sample 13 and the maximum was 14.3 for sample 21. On the average, the ascorbic acid content of the pasteurized milk was 11.0 ± 1.93 times its riboflavin content.

The ratio of the riboflavin to the thiamine content of the raw milk varied from sample 19 in which the riboflavin content was 4.1 times that of thiamine to sample 7 and 12 which contained 5.3 times as much riboflavin as thiamine. On the average the raw milk contained 4.6 ± 0.3 times as much riboflavin as thiamine, and the riboflavin content of the pasteurized milk averaged 4.9 ± 0.81 times that of thiamine.

The ratio of the ascorbic acid to the thiamine of raw milk was much higher than the ratio of riboflavin to thiamine or of ascorbic acid to riboflavin. The ratio of ascorbic acid to thiamine in raw milk ranged from 43.8 for sample 13 to 71.4 for sample 27 and averaged 59.8 ± 6.40. There was a larger loss of ascorbic acid than of thiamine during the pasteurization process, and the ratio ranged from 23.3 for sample 13 to 77.1 for sample 24. The average ascorbic content of the pasteurized milk was 53.8 ± 9.63 times that of the thiamine.

SUMMARY

The ratios to each other of ascorbic acid, riboflavin, and thiamine have been determined for winter milk produced under controlled conditions by the college herd of Ayrshire, Guernsey, Holstein, Jersey, and Shorthorn cows. The vitamin contents of the milk were determined for samples taken just previous to and immediately following pasteurization by the holding process for 30 minutes at 143°–145° F.

The ascorbic acid content of the raw milk ranged from 14.0 mg. to 22.5 mg. and averaged 19.7 ± 0.18 mg. per liter; after pasteurization in stainless steel equipment, the extreme values were 7.0 mg. and 19.1 mg., with an average value of 15.9 ± 2.7 mg. per liter.

The riboflavin content of the raw milk varied from 1.35 mg. to 1.75 mg. and averaged 1.51 ± 0.09 mg. per liter. The corresponding riboflavin values for the pasteurized milk were 1.19, 2.06, and 1.48 ± 0.01 mg. per liter, respectively.

The thiamine content of the raw milk varied from 0.29 to 0.35 mg. and averaged 0.33 ± 0.02 mg. per liter. Corresponding thiamine values for the pasteurized milk were 0.21, 0.34, and 0.30 ± 0.03 mg., respectively.

The ratios of riboflavin, ascorbic acid, and thiamine were computed for both the raw and the pasteurized milk. The raw milk contained 4.6 ± 0.3 times as much riboflavin as thiamine, 13.1 ± 1.43 times as much ascorbic acid as riboflavin, and 59.8 ± 6.40 times as much ascorbic acid as thiamine, On the average, the pasteurized milk contained 4.9 ± 0.81 times as much riboflavin as thiamine, 11.0 ± 1.93 times as much ascorbic acid as riboflavin, and 53.8 ± 9.63 times as much ascorbic acid as thiamine.

ACKNOWLEDGMENT

The authors express their appreciation to Mr. Elliott Greenwood and Prof. Clarence H. Parsons for supplying the samples of milk and data concerning the feeding and management of the cows.

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SOME RESULTS OF FEEDING RATS A HUMAN DIET LOW IN THIAMINE AND RIBOFLAVIN

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FIVE FIGURES

(Received for publication March 22, 1943)

A report of some of our results of feeding rats a human diet low in thiamine has been made (Higgins, Williams and Mason, '43). The present report covers the results obtained during a similar study wherein young rats of the same genetic strain were fed a human diet low in both thiamine and riboflavin.

The riboflavin content of the basal diet in our initial study was 2.5 μg. per gram. In order to reduce this level, certain changes were made in some of the constituents of the original basal diet. Skim milk powder, cheese and skim milk were omitted altogether. Larger portions of bread, beef roast and butter were allowed and cream was added. Except for these changes and the substitution of certain fruits and cereals the basal diet resembled that used before. Clinical results of providing this diet to human subjects have been reported (Williams, Mason, Cusick and Wilder, '43). The components of this basal diet are shown in table 1. Vitamins A and D, ferrous sulphate and tricalcium phosphate were added to the diet in amounts sufficient to provide not less than 10 International Units (I. U.) of vitamin A, 1.0 I. U. of vitamin D, 0.4 mg. of ferrous sulfate and 1.2 mg. of tricalcium phosphate per gram of diet.

The five diets tested are designated in this report as diets E, F, G, H and J. They differed from one another only in the flour from which the bread component of each diet was prepared. In diet E (table 1) the bread was prepared with a patent white flour; in diet F, the patent white flour was restored in content of thiamine to the approximate level of

TABLE 1

Biochemical data on diets used in experiment.

ANALYSIS OF DIET E	Carbohydrate	(%):	68.6
	Protein	(%):	13.1
	Fat	(%):	16.5
	Calories per gram:		4.7
	Calories from carbohydrate	(%):	56.1
	Calories from protein	(%):	13.3
	Calories from fat	(%):	30.6

DIET	Per gram of dry diet	
	Thiamine	Riboflavin
	μg.	μg.
E — Basal diet	0.96	2.00
F — Basal diet supplemented with thiamine	1.62	2.00
G — Basal diet suppplemented with thiamine and riboflavin	1.82	2.90
H — Basal diet supplemented with thiamine, riboflavin and niacin [1]	1.78	2.90
J — Basal diet (bread component of whole wheat)	1.94	2.60

[1] 6 mg. of niacin in each pound of flour.

whole wheat flour; in diet G, the patent white flour was restored in content of thiamine and riboflavin to the approximate levels of whole wheat flour; in diet H, the flour was identical with that of diet G except that niacin was added in amounts equal to 6 mg. per pound (0.5 kg.) of flour; and in diet J the bread was prepared with whole wheat flour. Each diet when thoroughly mixed weighed 1,087.8 gm. and, when

dried at a temperature of 65° C. for consumption by rats, weighed 465 gm. When ready for use, assays were made for the thiamine and riboflavin contents of the dried diets. We used the methods of Hennessy ('41) and of Conner and Straub ('41) for these determinations. The results of these assays are summarized in table 1.

Young rats, weighing about 80 gm., were used for this study. They were housed in metal cages with a screen bottom, four to a cage; food and water were always available. Eight animals were fed each of the diets tested and observations were continued for 14 weeks. All animals were weighed each week. Beginning at the end of the eleventh week, and for 10 days threafter, the average intake of food and water per day was recorded. Diets were provided in containers, all waste was salvaged and the change of weight was recorded as food consumed. Likewise water, provided in bottles attached to the cages, was measured daily and the decreases of volume were recorded as cubic centimeters consumed.

At the end of the twelfth week, samples of heart blood were withdrawn for study of the number and size of the erythrocytes, the concentration of hemoglobin, the percentages of reticulocytes and the number of leukocytes. At the end of the experiment (14 weeks), all animals were killed and portions of the livers, thyroids and pituitaries were fixed in Zenker-formalin solution and appropriately prepared for histologic study. Analyses of the concentrations of thiamine and riboflavin per gram of tissue were made of the livers, kidneys, skeletal muscles and testes of five animals which had eaten the basal ration for 14 weeks. These data were contrasted with analyses made of these tissues from five animals which had eaten our standard laboratory ration for a comparable period.

RESULTS

Body weight. Marked differences in the growth rates of these groups of rats are recorded in figure 1. These weight data confirmed our earlier observations that fortification

of flour with thiamine had only a slight influence on the growth rate. Fortification with thiamine and riboflavin improved the diet sufficiently to promote greatly increased growth rates. The addition of niacin was of only slight value and the conclusion is indicated that fortification of the flour with these three vitamins did not promote growth rates nearly as satisfactorily as the substitution of whole wheat flour for the patent flour.

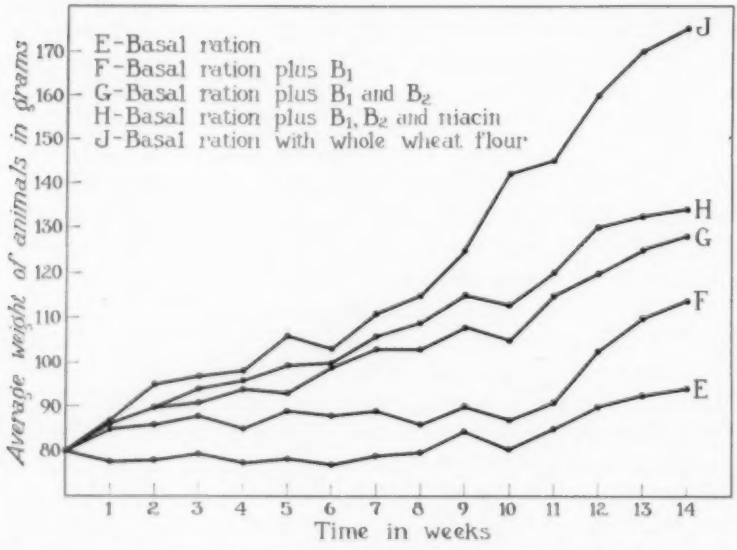

Fig. 1 Growth curves of five groups of rats fed the human diets in which the only variable was the flour from which the bread component was made.

Intake of food and water. The data on intake of food and water over a 10-day period near the close of the experiment are assembled into table 2. They show that rats eating the basal diet were taking 5.7 gm. of food (26.8 cal.) per day and yet their weights remained essentially constant. When the thiamine was added, the intake of food increased to an average of 40.0 cal. per day and yet the average gain was less than 1 gm. a day. Even animals eating diet J, consuming

42.7 cal. per day, gained on the average only 1 gm. a day during the test period. When the food intake per unit of surface area (table 2) was computed, animals eating diet J were taking less food than those eating diet H and yet their body weights were considerably greater.

Although appetites were apparently good and the caloric intake was adequate to promote better growth, effective utilization of the foods apparently was not obtained in any of

TABLE 2

The intake of food and water over a 10-day period and the blood findings.

CATEGORY OF INTEREST		DIET				
		E	F	G	H	J
Food intake: gm./rat/day		5.7	8.5	7.7	9.6	9.1
Food intake: gm./100 sq. cm. surface area/rat /day		3.1	4.5	3.5	4.3	3.6
Water intake: cc./rat/day		7.7	9.0	11.3	11.0	13.9
Blood	Erythrocytes: millions/cu. mm.	7.46 ± 0.19 [1]	7.81 ± 0.24	8.76 ± 0.16		8.85 ± 0.11
	Hemoglobin: gm./100 cc.	12.5 ± 0.34	13.5 ± 0.21	14.0 ± 0.14		15.1 ± 0.30
	Reticulocytes: %	3.0 ± 0.27	2.9 ± 0.38	1.5 ± 0.16		0.9 ± 0.09
	Leukcoytes: thousands/cu. mm.	9.3 ± 0.89	10.9 ± 0.65	10.17 ± 0.47		17.03 ± 1.21

[1] Standard error.

the groups. This may be due to the low riboflavin content of all the diets which were tested, even diet J (2.6 μg. per gram). These rats, consuming on the average 9.1 gm. of food per day, were getting but 23.6 μg. of riboflavin daily. In our initial study, very much better growth than in the present study was obtained among animals eating the ration containing whole wheat flour, wherein the intake of riboflavin was about 35.0 μg. daily.

Observations on the blood. The data assembled from blood samples at the end of the twelfth week are condensed in table 2. The means of the determinations together with their standard errors are included. Hypochromic anemia developed in animals which ate diet E. The addition of thiamine alone improved the erythrocyte and hemoglobin levels somewhat, although not significantly so, but the addition of both thiamine and riboflavin stimulated production of erythrocytes, resulting in an average increase of 1.30 ± 0.24 million cells per cubic millimeter of blood. This difference is more than five times the standard error. Likewise the concentration of hemoglobin per 100 cc. of blood increased by 1.5 ± 0.36 gm. when animals ate diet G. Diet J, which contained whole wheat bread, did not increase the number of erythrocytes, but did significantly increase the homoglobin levels over those of diet G. These data confirm our earlier observation, as well as those of others, that the addition of riboflavin, as in diet G, stimulated production of erythrocytes and hemoglobin.

The liver. The livers of all animals which ate diet E showed extensive peripheral degeneration throughout most of each hepatic lobule (fig. 2). The cells were necrotic; they contained extensive vacuoles and their nuclei were pyknotic. Appropriate stains revealed varying amounts of fat droplets, indicating, presumably, a fatty degeneration in the peripheral portions of the lobules. Fortification of the flour with thiamine seemed to improve somewhat the appearance of hepatic cells around the central veins, and the increment of riboflavin was of further value. Among animals eating diet J a nearly normal hepatic cellular organization was maintained (fig. 3). Some peripheral necrosis developed but fat stains did not indicate degenerative changes. Fatty livers, or hepatic cirrhosis such as occurred among animals fed diets low in choline (Best, '41; Blumberg and McCollum, '41; Spellberg, Keeton and Ginsburg, '42) with high fat and low protein percentages, did not develop among our animals. When rats are fed synthetic diets, low in casein but not necessarily high in fats, hepatic injury is produced consistently (György, '42).

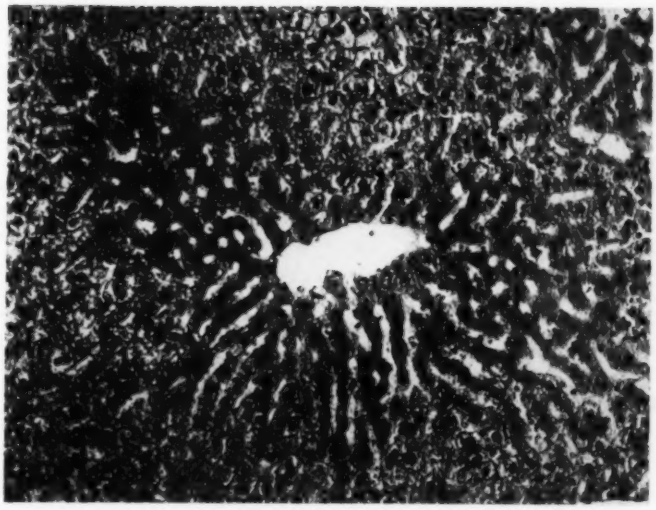

Fig. 2 Sections of liver of animal eating diet E (× 165). Peripheral necrosis of the hepatic cells had developed.

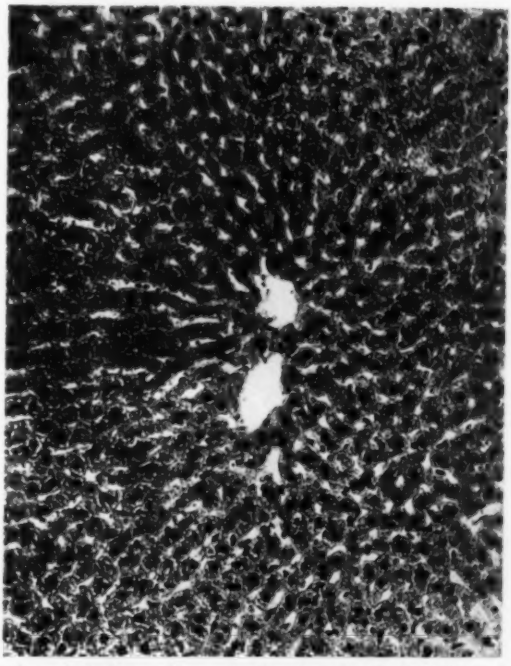

Fig. 3 Section of the liver of an animal eating diet J (× 165). A more nearly normal type of hepatic architecture was produced.

High casein ratios or the addition of methionine and choline exerted a considerable protective influence. The hepatic injuries observed in our rats fed these so-called human diets may be due in part to the protein-fat ratio (13.1 : 16.5). Although this percentage of fat (16.5) is not high, it is appreciably higher than that of our low thiamine human diet (10.8)

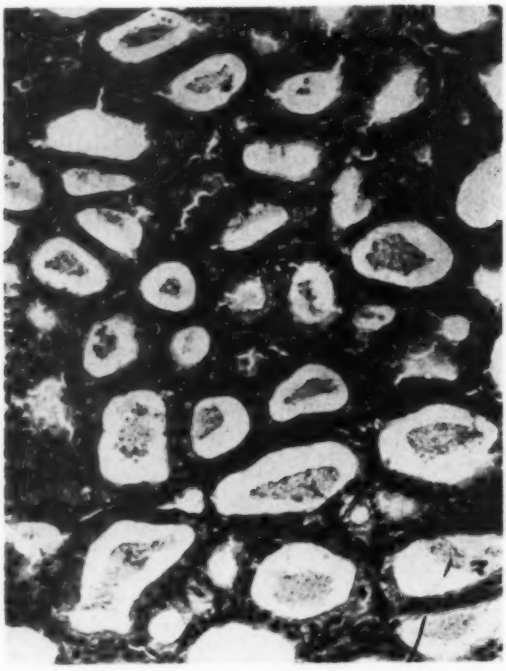

Fig. 4 Section of thyroid gland of animal eating diet E (× 200). Marked hyperplasia of the acini, with atrophic colloid masses, is shown.

and four times the fat percentage of our standard laboratory rat ration, in which the protein-fat ratio is 22:4.

The thyroid gland. Thyroid glands of all animals eating the experimental diets were hyperplastic (fig. 4). The follicles contained small amounts of colloid which was often contracted into amorphous masses and was stained blue with

Koneff's stain. Infrequently, the marginal colloid of such follicles had a reddish tinge. An acidophilic colloid was always observed in the thyroid glands of the control animals fed our standard ration. The follicular epithelium was more of the columnar type, often obliterating the lumina, in contrast to the normal flat or cuboidal type. The average height of the follicular cells in the thyroids of animals eating diet E was 8.2 microns; of those eating diet G, 12.7 microns; and of those eating diet J, 11.8 microns. The degree of hyperplasia

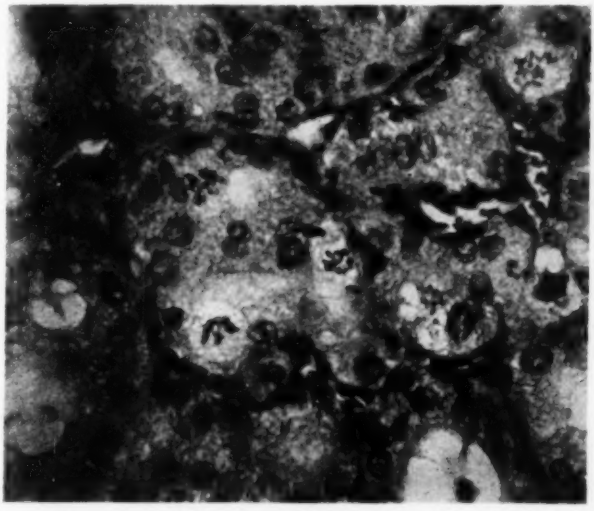

Fig. 5 Section of thyroid gland of animal eating diet G (× 450) Excessive hyperplasia was produced. Acinar lumina were obliterated and mitotic figures were common.

is apparent when it is recalled that the average height of cells in follicles of normal thyroids, in animals fed a standard ration was about 5.0 microns. On the contrary, even greater stimulation occurred in thyroids of animals eating diet G than occurred in those eating diet E. Cellular hypertrophy was sufficiently marked to occlude the lumina of the acini completely in the G group, and mitotic figures were abundant (fig. 5). The iodine content of the diets was not determined

but, since the salt used in the preparation of all diets was iodized, we have reason to believe that the iodine intake was probably adequate.

The pituitary gland. Differential counts of the cells in sections of the pituitary gland of some of the animals are shown in table 3. The data, while inconclusive, suggest that these deficient diets may have caused a reduction in the percentage of acidophilic cells and a corresponding increase in the percentage of chromophobes. The slight changes in the percentages of basophilic cells are not considered to have any special significance. Apparently the diets were incapable of maintaining the normal secretion of growth hormone. The addition of thiamine and riboflavin to the diets did not restore normal cell distribution in the pituitary.

TABLE 3

Percentages of cell types in the anterior lobe of pituitary.

DIET	ACIDOPHILS	BASOPHILS	CHROMOPHOBES
Laboratory ration	45.4	4.5	50.1
E	37.4	3.8	58.7
G	38.7	3.4	57.8
J	39.8	5.2	55.0

Vitamin assay of the tissues. Using the method of Hennessy ('41) for thiamine and that of Conner and Straub ('41) for riboflavin we determined the concentration of these vitamins in the liver, skeletal muscle, kidney and testes of five animals which ate diet E, and five which ate our standard ration for 6 months. These data are assembled in table 4. Concentrations of both thiamine and riboflavin in all tissues examined were significantly lower among the animals fed diet E than among the controls except for the riboflavin levels of the skeletal muscle and testes, and in these tissues the levels were statistically alike. Schultz, Light, Cracas and Atkin ('39) determined the thiamine concentration per gram of certain tissues of rats fed a synthetic diet for 28 days supplemented daily by (1) small amounts of thiamine (15 μg.) and (2) large amounts of

thiamine (215 μg.). Their group given the larger daily supplement of thiamine (215 μg.) had, after 28 days, 10 μg. of thiamine per gram of liver, 1.3 per gram of muscle, 6.7 per gram of kidney and 6.6 per gram of testes. These data correlate well with our own control data (table 4). In studies at the University of Texas ('41) concentrations of thiamine and riboflavin per gram of dry tissue were determined for the normal rat, microbiologic tests being used for the assays. When corrections are made for moist weights, our data, with few exceptions, compare well with those of these workers.

TABLE 4

Concentrations of thiamine and of riboflavin in tissues of conrol rats and of rats fed a diet low in thiamine and riboflavin.

ORGAN	THIAMINE CONCENTRATION μG./GM.		RIBOFLAVIN CONCENTRATION μG./GM.	
	Control	Diet E	Control	Diet E
Liver	9.7 (8.7 — 10.6) [1]	2.7 (2.2 — 3.1)	25.6 (23.2 — 28.8)	11.7 (10.0 — 14.6)
Skeletal muscle	1.6 (1.5 — 1.7)	0.44 (0.36 — 0.49)	1.0 (0.92 — 1.1)	1.2 (1.1 — 1.4)
Kidney	6.5 (5.9 — 7.7)	1.9 (1.6 — 2.1)	29.2 (27.9 — 32.5)	22.2 (18.3 — 24.2)
Testes	7.2 (6.6 — 8.1)	4.9 (3.9 — 5.6)	2.7 (2.4 — 3.2)	3.1 (2.7 — 3.6)

[1] Figures in parentheses indicate range of values.

SUMMARY AND CONCLUSIONS

This report covers some of the results of a study of feeding white rats a human diet, composed of foods commonly appearing on American tables, wherein the thiamine and riboflavin contents were low.

Five diets, differing from one another only in the flours from which the bread component was made, were prepared. Supplementations of patent white flour with thiamine, riboflavin and niacin, while improving the growth rate of the rats, were not adequate to secure weights attained by animals eating the diet that contained whole wheat flour.

The addition of thiamine to the flour increased the daily caloric intake from 26.8 to 40.0 and yet the average gain of weight was less than 1 gm. per day for the period when the intakes of food and water were tested. The low daily intake of riboflavin was inadequate for satisfactory growth.

The addition of both thiamine and riboflavin to the diet prevented the hypochromic anemia which developed among rats which ate either diet E or diet F. Essentially normal blood values were obtained by fortification of the flour with these two vitamins as well as by the diet which contained the whole wheat flour.

Peripheral necrosis associated with mild fatty degeneration occurred in the livers of all animals eating diet E. Cirrhosis did not develop. The addition of thiamine and riboflavin appeared to prevent extensive necrosis but the livers of rats eating the diet that contained whole wheat flour had a more nearly normal cellular organization than those of rats to whose diet thiamine and riboflavin were added.

The thyroid glands of all animals were hyperplastic. Mitotic figures were common in the acini of the thyroids of animals eating diet G. Extensive cellular activity was demonstrated even in animals eating diet J. These changes may be due, not to any lack of vitamins or to inadequacy of iodine, but perhaps to some dietary imbalance.

Changes from the normal in the percentage distribution of cells in the pituitaries were observed in these animals. These changes constituted relative increases of the percentages of chromophobes and corresponding decreases of the percentages of acidophils.

Chemical analyses of the concentrations of thiamine and riboflavin of the livers, skeletal muscles, kidneys and testes of animals eating diet E were made. These values showed excessively low concentrations of these vitamins in the tissues analyzed. They compared favorably with such data assembled elsewhere.

Fortification of the flour used in the bread component of the low thiamine-low riboflavin human diets with thiamine, ribo-

flavin and niacin in the amounts indicated proved inadequate to promote satisfactory growth or to prevent pathologic changes in the livers, thryroids and pituitary glands of white rats.

ACKNOWLEDGMENT

All vitamins used in this study were graciously supplied by Merck and Company. It is a pleasure to express our gratitude to them for their continued interest in our studies.

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THE ROLE OF BIOTIN AND "FOLIC ACID" IN THE NUTRITION OF THE RHESUS MONKEY [1]

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FOUR FIGURES

(Received for publication April 9, 1943.)

INTRODUCTION

It has been pointed out in a previous paper (Waisman et al,, '43) that young monkeys (macaca mulatta) fail to grow and develop on a diet consisting of sucrose, purified casein, mineral salts, corn oil and cod liver oil together with adequate levels of thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, choline, para aminobenzoic acid (PABA) and i-inositol. On such a diet there is a gradual loss of weight followed by a syndrome of anorexia, leucopenia, lowered resistance to secondary infections, increasing cachexia and death. When liver extract, whole liver or a "solubilized liver" (commercial enzymically hydrolyzed) preparation was included with the "synthetic" diet, remission of all symptoms was noted and the animals showed striking weight gains. The complete absence of symptoms in those monkeys receiving the liver diets for 2 years is evidence that some factor (or factors) in the liver preparations was active in preventing the deficiency

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station and aided in part by a grant from the National Foundation for Infantile Paralysis, Inc.

We are indebted to Merck and Co., Rahway, New Jersey, for the synthetic vitamins; to Abbott Laboratories, North Chicago, Ill. for halibut liver oil; and to Wilson Laboratories, Chicago, Ill. for the various liver products.

syndrome in the animals. In this report evidence is presented that both biotin and the norite eluate factor (Hutchings et al., '41) or, as it is also known, "folic acid" (Mitchell et al., '41), are at least two factors required by monkeys in addition to the known crystalline and readily available vitamins.

EXPERIMENTAL

The diets fed throughout these studies together with the methods used for the care and handling of the animals have already been described (Waisman et al., '43). The weights of the animals together with daily observations of each animal were the guides used in determining when therapy was indicated. The animals which lost weight and failed to drink the vitamins or the particular concentrate were given the material by stomach tube until sufficient improvement resulted so that the supplement was consumed voluntarily.

The biotin used in these experiments was a commercial concentrate [2] containing 20 μg. per milliliter. The "folic acid" concentrate was the norite eluate of liver prepared according to the method of Hutchings et al., ('41) through the first norite absorption and elution with the ammonia-alcohol mixture. Each preparation of the concentrate was microbiologically assayed [3] by the Streptococcus lactis method (Mitchell and Snell, '41) and the activity expressed as percentage potency of a standard solubilized liver. The majority of the preparations ranged in activity from 35 to 65%, but an occasional preparation was only 20% as active as solubilized liver. The various liver products and liver concentrates were also microbiologically assayed both for their biotin [4] and "folic acid" content.

The growth data were obtained with young monkeys who were initially depleted of their stores of the various food factors by giving them the basal synthetic diet with the known crystalline vitamins until the first constant drop in weight was

[2] S.M.A. Co., Chagrin Falls, Ohio, no. 200.
[3] By Mr. T. D. Luckey.
[4] By Mr. D. Miller.

observed. They were then given the "folic acid" concentrate daily in the solution containing the vitamins for periods of a week to 2 months or more until a definite conclusion could be reached as to its effectiveness as a growth-promoting concentrate. Control animals received the material from the beginning. Red and white cell and hemoglobin determinations were made at convenient times, commonly every 2 weeks, upon blood samples drawn from the marginal veins of the ears. The white cell counts were made at the same time daily during the deficiency and throughout the period of "folic acid" therapy.

Biotin studies

It has been shown (chart 2, Waisman et al., '43) that the addition of 20 μg. biotin per day to the synthetic ration did not prevent the decline in weight nor improve the condition of the animal in any way. While no growth effects can be attributed to biotin under the experimental conditions employed, the results appear convincing that this vitamin is concerned in some way in the maintenance of normal fur.

A total of eleven monkeys were used in experiments involving biotin. Some monkeys received 20 μg. from the beginning of the experiment, several received the biotin only after nutritional failure became apparent, and others received the biotin together with various liver fractions. The five animals which received 20 μg. biotin per day together with the nine crystalline vitamins and the synthetic diet from the start showed the same syndrome of nutritional failure as though the animals were fed the diet and the nine vitamins without biotin. These animals declined within the 30–85 days noted previously; similarly, several other animals which received the biotin when the weight curve showed a plateau showed no delay in the onset of nutritional failure. It is apparent that biotin is not the factor which maintains monkeys fed the synthetic diet, nor does it appear to be concerned in delaying the symptoms previously described when no other fractions were fed.

In our previous paper the experimental procedures were described under which we kept twelve monkeys in excellent nutritive condition for nearly 2 years on synthetic diets containing 3% liver extract. In these monkeys the fur was normal in texture, color, quantity, and brilliance. In contrast to this, the monkeys maintained on 3% solubilized liver gradually lose their fur color, then lose areas of fur leaving a denuded portion in which there may be occasional dermatitis, and finally

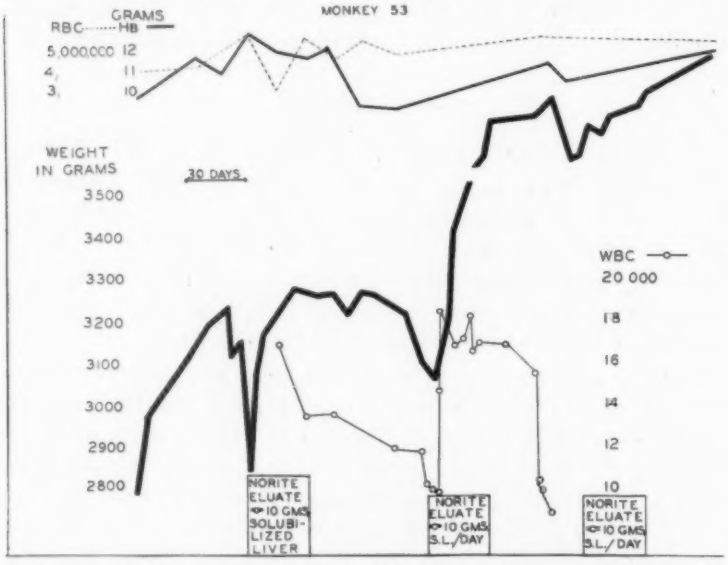

Fig. 1 Typical growth responses and white blood cell increase following the administration of a norite eluate of solubilized liver ("folic acid" concentrate).

the entire body surface will be largely denuded and the monkey will have a blanched appearance with the skin markings clearly visible through the short guard hairs that remain. These gradual changes are shown in figures 2, 3, and 4. The time of onset of this denudation and off color appearance of the fur is variable, apparently dependent upon the quality of the diet fed prior to the experimental period in question. The six monkeys fed 3% solubilized liver became denuded in from 2 to 7 months.

The administration of 20 μg. of biotin per day to the denuded animals resulted in a gradual return of the fur; within 30 days definite areas were filled with long hairs and after 60 days much of the color returned. In several monkeys after 4 months of biotin therapy the fur was completely restored but the

Figure 2

Various stages in the loss of fur in monkeys receiving the solubilized liver diets are illustrated in this figure, as well as figs. 3 and 4.

original color was not attained. It is not possible to say at this time whether another factor is involved but it should be remembered that in those monkeys kept on the basal diet plus 3% liver extract, the fur retained its normal golden brown color; it should also be noted that together with the loss of hair and a definite dry dermatitis, a "rusty" appearance of the

skin appeared either prior to the alopecia or concurrently with it. This "rusty" condition is very likely due to the porphyrin-type compound described as the material in the "bloody tears" of pantothenic acid deficient rats (McElroy et al., '41). In those monkeys fed biotin, this "rusty" condi-

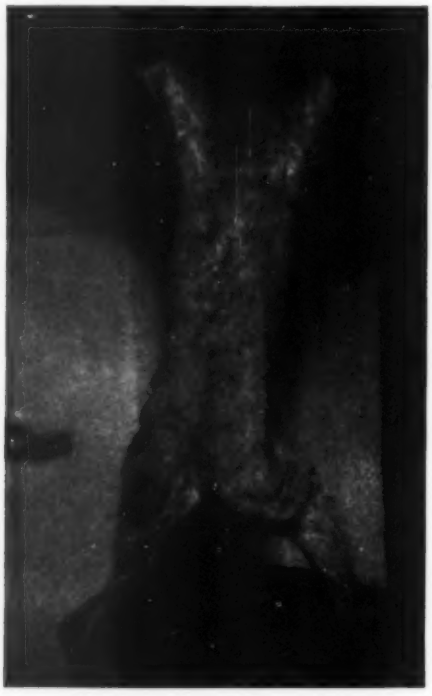

Figure 3

tion completely disappeared shortly before the hair began to grow back. The alopecia develops either during good growth on diets containing 3% of solubilized liver or during poor growth and even weight loss on diets containing 1% of the same. To illustrate, a monkey getting 1% solubilized liver showed discolored fur and denudation long before the loss in weight which occurred because of the inadequacy of "folic

acid'' (to be described later) on the 1% level of solubilized liver. Biotin feeding was started 2 months after the monkey was placed on the 1% solubilized liver diet and the fur began to grow in slowly although the monkey showed a gradual loss in weight. At the time the diet was changed to 3% solubilized

Figure 4

liver the fur was nearly normal in amount although still a little off color in spots.

An interesting observation was made on two animals which received 1% solubilized liver diet but no added pantothenic acid. After a short period of growth, the animals gradually lost weight and small areas of baldness were visible on the sixty-ninth day when a ''folic acid'' concentrate equivalent to 10 gm. of solubilized liver was fed to prevent further weight

loss. The first animal began to grow again but became increasingly denuded until, after 60 days more, it was completely lacking in hair. Continuing the "folic acid" supplement, growth was nearly normal and, 95 days after the greatest hair loss was first observed, a slow growth of hair had begun. In another 30 days the hair growth was quite profuse with only a few bald patches remaining. The color was abnormal and the texture faulty. It should be remembered that at no time did this monkey receive biotin. The other monkey paired on this experiment also lost its fur but in a slightly longer period of 150 days. This animal was then fed 3% solubilized liver, the pantothenic acid still being omitted from the daily supplement, and although the weight of the animal was increased over that gained on 1% solubilized liver, the fur continued to be sparse and only a portion of the shoulder was covered with fur of a lighter color. The "folic acid" supplied by the 3% solubilized liver appears then insufficient to give the fur growth that the "folic acid" concentrate supported. This assumption is borne out by the fact that animals getting 3% solubilized liver become denuded while those animals given "folic acid" concentrate equivalent to 10% solubilized liver did not show this denudation during the period of our experiments. It is noteworthy that one of our monkeys fed the 3% solubilized liver diet but getting no pantothenic acid for more than 15 months has shown no change in fur color or texture.

"Folic acid" results

An appraisal of our data early in 1942 after 8 months of feeding a variety of diets led us to believe that there was a difference in the growth of monkeys fed the synthetic diet and all nine crystalline vitamins and those fed varying amounts of liver preparations, grass extracts and a ration containing heated corn and wheat middlings (Waisman et al., '42). Liver extract was chosen as the starting product for fractionating work in an attempt to identify the active factor or factors. It was found that the factor was stable to alkali, not extracted by prolonged continuous ether extraction in acid solution, ad-

sorbed by norite and eluted with alcohol and mild alkali. With these data in mind and with the aid of microbiological assays of our diets and a knowledge of liver preparations and fractions which had accumulated in this laboratory, we were led to the conclusion that the factors in liver and grass were probably identical with the factor called "norite eluate factor" by the Wisconsin group (Hutchings et al., '41) and therefore with the factor named "folic acid" by the Texas group (Mitchell et al., '41). The feeding of a concentrate of the norite eluate to monkeys which showed the weight loss characteristic symptoms (leucopenia, anorexia, etc.) showed first an increased appetite which reflected itself in definite weight gains and, second, a sharp increase in white blood cells to the normal level for the monkey.

At this writing more than twenty-five trials with at least fifteen monkeys fed the "folic acid" concentrate have convinced us that this preparation contains a factor or factors essential for normal growth in this animal. In most of our trials the equivalent of 10 gm. of solubilized liver was fed, but we have obtained equal responses in several monkeys with an equivalent of 5 gm. per day. The response to the "folic acid" concentrate was considered reliable since in a sufficient number of animals the basal diet was first fed until nutritional failure resulted and this was followed by the feeding of either the 3% liver extract or 3% of the solubilized liver diets. After an adequate response was obtained the animal was returned to the basal synthetic diet until nutritional failure again resulted and then the "folic acid" concentrate was given. This procedure was reversed in several cases, the "folic acid" being given first and followed by either the 3% liver extract or solubilized liver.

That 1% solubilized liver supplies inadequate "folic acid" for growth and maintenance is shown by the nutritional failure and by the response which can be obtained in those animals when they are fed a "folic acid" concentrate equivalent to 5% liver. The feeding of the "folic acid" concentrate to an animal already cachectic and moribund delayed the time of death for

a week or 10 days beyond that period found previously. Although life was prolonged, the hemorrhagic condition of the digestive tract due to the dysentery may have accounted for the inability of the animal to absorb fully and therefore respond to the supplement.

Monkeys fed from the beginning with "folic acid" concentrate equivalent to 5 or 10 gm. of solubilized liver together with the crystalline vitamins and the synthetic diet, have shown good growth for more than 130 days and are still on experiment. Other monkeys have been given the "folic acid" concentrate with 20 μg. of biotin from the start and have been maintained now for more than 266 days. This type of experiment is indicative of the adequacy of the supplements to the synthetic diet; also of the adequacy of the "folic acid" concentrate to supply the essential factor or factors for maintaining normal growth and well-being beyond the time when the animal would ordinarily be expected to fail if it were given the synthetic diet alone. In order to gain additional information on the specific factors furnished by the "folic acid" concentrate, it will be necessary to attempt further fractionation and isolation procedures.

Along with the response in growth, the second effect of the "folic acid" supplement has been to restore the normal blood picture in the deficient monkey showing leucopenia. The daily white cell count was followed during most of the "folic acid" trials in the monkeys, together with the counts in those monkeys which served as controls (those getting 3% solubilized liver described in this and the preceding paper). As will be seen in figure 1, typical responses of white blood cell formation have been obtained following "folic acid" (norite eluate) therapy. The white cell increase follows very closely the feeding of the "folic acid" containing supplements. Each monkey must necessarily serve as its own control since the normal white cell count varies from animal to animal, as found by Shukers et al. ('38) and confirmed in this laboratory. The removal of the "folic acid" supplement resulted in the gradual loss in weight, but it appeared that the white cell count

dropped before extreme deficiency symptoms developed. No differential counts were made in this study since we were interested in the total response which could be elicited by the "folic acid" concentrate. Wilson et al. ('42) found that a commercial "folic acid" concentrate was effective in establishing a normal white cell equilibrium in two monkeys.

DISCUSSION

The growth response which has been noted in deficient monkeys when a "folic acid" concentrate was fed is adequate demonstration that this preparation contains the factor or factors found in the three liver products previously described. The curative trials in which the "folic acid" was fed in amounts equivalent to 10 gm. solubilized liver gave as effective responses as the 3% original solubilized liver. This appears valid since the norite eluates contained on the average approximately 40–50% of the original activity, and the monkeys consumed approximately 200 gm. of the 3% liver diet. The "folic acid" supplement aided in overcoming the dysentery fully as well as the original liver products in the deficient animals. Since the white cell count increased consistently upon the addition of the "folic acid" to all deficient monkeys, it is convincing evidence that this concentrate contains the factor responsible for the remission of the leucopenia in monkeys fed liver.

Experiments are still in progress in which the "folic acid" is the only supplement other than the nine crystalline vitamins, fed from the beginning to monkeys; however, these experiments have gone far enough to support the conclusions drawn. Although these animals were not fed biotin, the data so far obtained indicate that the monkeys are able to grow on the synthetic diet alone and be maintained in apparent good health for a period longer than the maximum time at which nutritional failure can be expected.

Monkeys fed 1% liver extract are able to grow and survive in contrast to those which fail on diets containing 1% solubilized liver. This observation may be important since it not

only points out the differences in the two liver products but also furnishes some evidence that liver extract supplies the necessary nutrients for the favorable intestinal bacteria. This notion is supported by the finding that monkeys, failing on the 1% solubilized liver diet and then fed the "folic acid" concentrate, are able to recover and resume growth, thus indicating that 1% solubilized liver does not furnish sufficient quantities of the factor. At the 3% level, adequate amounts of the factor(s) are supplied so that there are no apparent discrepancies in the growth between the animals getting 3% solubilized liver and 3% liver extract.

There are at present no means of determining with any finality that the "folic acid" concentrate as prepared contains only the one physiologically active compound named "folic acid". It is difficult at this time to refer the growth response, the remission of the leucopenia, and perhaps also the prevention of the dysentery to only one substance in the liver concentrate, but until pure compounds are isolated and shown to be physiologically active, it appears unwise to attribute each observation to a separate substance.

The question of fur loss calls attention to several noteworthy points. With but one exception in nearly twenty animals receiving 3% liver extract, all the monkeys showed normal fur texture and color. Liver extract fed as low as 1% was able to maintain normal fur and skin structure. With 1% solubilized liver however, the animals became completely blanched and denuded. The gradual loss of fur and final denudation were independent of body growth in monkeys as shown by the continued growth of those animals getting 3% solubilized liver. The gradual return of some fur was noted after 3 months in one animal given the 1% solubilized liver diet and "folic acid" concentrate, although no biotin was fed. Another monkey paired on this experiment also lost all its fur but had not received any "folic acid" concentrate and at this writing remains nearly completely denuded. If the role of biotin is a specific one, then this study, showing the necessity of supplying this factor to monkeys for normal skin and fur structure,

is one of the first experiments in which raw egg white is not used to demonstrate that experimental animals require biotin. In this laboratory, Cooperman, Waisman and Elvehjem ('43) have presented evidence that hamsters require biotin when fed a diet containing no raw egg white. Sydenstricker et al. ('42) and Oppel ('42) have reported on biotin studies in man.

Some quantitative relationships of the factors involved are brought into focus when one considers the denudation which occurs even in those animals receiving 3% solubilized liver which ostensibly furnishes adequate "folic acid" for growth while the "folic acid" equivalent to 10% solubilized liver stimulates fur growth in monkeys getting the 1% solubilized liver diet. The solubilized liver and liver extract contain approximately the same quantity of "folic acid" but the biotin in liver extract is about five times that which occurs in solubilized liver. Solubilized liver then contains not only insufficient biotin but also an inadequate quantity of the factor in liver extract which may be necessary for the formation of biotin by intestinal bacteria. The exact level of biotin necessary for fur replacement is not known since up to this time we have used only the 20 μg. per day supplement. The possible role of the interrelationship of the various vitamins in influencing the growth of intestinal bacteria, which in turn synthesize known and unknown factors then available to the animal, is shown by the finding of a normal skin and fur in a monkey getting no pantothenic acid other than the small amount supplied in the 3% solubilized liver diet. The ability of biotin to cause this regrowth of fur is clearly evident, but the role of other factor(s) in either stimulating synthesis of biotin or in supplying other substances per se is not fully established.

SUMMARY

1. The nutritional failure which results in monkeys fed a "synthetic" diet plus eight crystalline members of the B group of vitamins and ascorbic acid can be readily cured or prevented by the feeding of a norite eluate fraction of liver, also called "folic acid".

2. Normal growth is observed in monkeys given only the "folic acid" concentrate in addition to the basal diet and the nine crystalline vitamins.

3. The leucopenia in the deficient monkeys is quickly alleviated by the administration of the "folic acid" preparation.

4. Monkeys kept on 1 or 3% solubilized liver for long periods show loss of hair and nearly complete denudation together with a slight dermatitis and "porphyrin-like" secretion on the skin. These changes are alleviated by the administration of biotin.

5. Biotin has no growth-stimulating properties under the experimental conditions described.

ACKNOWLEDGMENT

Dr. Paul F. Clark aided greatly in the revision of this manuscript.

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LOSSES OF VITAMINS IN LARGE-SCALE COOKERY [1]

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(Received for publication March 22, 1943)

Very little work has been done on vitamin losses in large quantity cookery. The estimated loss of thiamine and ascorbic acid in the restaurant cooking and holding of vegetables is usually given as about 75% (Nagel and Harris, '43).

The present study was conducted at one of the cafeterias feeding industrial workers at the Boooklyn Navy Yard. The purpose of this study was to give a rough estimate of the losses in vitamins in the actual field of large-scale cookery. It is in no sense a carefully controlled study to locate the steps in the cookery that cause these losses.

Most of the vegetables used in this cafeteria were frozen — usually green lima beans, green string beans, spinach and green peas. Raw vegetables used were potatoes, carrots and turnips; canned vegetables were mostly corn and sauerkraut. Canned beets and carrots were used occasionally.

Table 1 gives the vitamin values per 100 gm. of the material as purchased, during the months of November and December,

[1] These studies were made possible through the cooperative efforts of Dr. E. S. Rogers, Director of the New York State War Council Office of War Nutrition Services, Mr. Walter Button of the Canteen Food Service, and Lt. Commander Hubbell, U.S.N.R. The thiamine determinations by the thiochrome method were run at Fordham University by M. Soodak and L. J. Vinson under the direction of Prof. L. R. Cerecedo. Thiamine determinations by the fungus assay method were run by Helen Adolph, and the riboflavin and niacin assays by Regina Machata, at the U. S. Plant, Soil and Nutrition Laboratory at Cornell University. The project was financed jointly by the U. S. Nutrition Laboratory, the New York State War Council and the Brewing Industry Foundation. Yeast was furnished through the courtesy of Mr. Hugh Harley, Secretary of the Brewing Industry Foundation.

1942. The values on the vegetables compare very favorably with those already reported, with the exception of cabbage. Frozen lima beans gave variable results. However, the beans were not consistent in either color or size, hence the wide variation in values. For example, the ascorbic acid varied from 3.9 to 15.4 mg. per 100 gm.; the vitamin A from 33 to 316 I.U. per 100 gm.

TABLE 1

Vitamin values for raw and frozen vegetables used at the Brooklyn Navy Yard, per 100 gm.

VEGETABLE	CAROTENE	THIAMINE	RIBOFLAVIN	NIACIN	ASCORBIC ACID
	Expressed as I.U. of vitamin A	*µg.*	*µg.*	*µg.*	*mg.*
Peas, frozen	500–566	332, 368 303	120	2.36	11.1, 11.4
Lima beans, frozen	33, 316	119, 79, 110, 85	64, 80 100	0.85, 0.82, 1.34	13.1, 4.5 3.9, 15.4
Green beans, frozen	83, 550	56, 73, 86, 96	100, 100 130	0.26, 0.42, 0.52	4.0, 9.8, 11.5
Spinach, frozen	9280	90, 101, 114	190	0.49	20.8, 34.9
Carrots, raw, old	9283	82	50	0.48	5.3
Potatoes, raw	neg.	53, 71, 82 82, 90, 62	30, 30	0.32, 0.54	8.7, 15.0
Cabbage, raw	neg.	71	30	0.27	4.3

The carotene, niacin, riboflavin and ascorbic acid values were determined at the U. S. Plant, Soil and Nutrition Laboratory at Cornell University. Carotene was determined by the chromatographic method (Moore and Ely, '41; Strain, '38; Curtis, '42), niacin by the microbiological method of Snell and Wright ('41), riboflavin by the microbiological method of Snell and Strong ('39), and ascorbic acid by the chemical method with a modification described by Morell ('41); the aliquot, however, was titrated to an end point with standard-

ized dye. Thiamine was determined by two different methods. Duplicate samples were sent to Fordham and Cornell Universities. Dr. L. R. Cerecedo of Fordham used the thiochrome method of Hennessy and Cerecedo ('42); the laboratory at Cornell used the fungus assay method first described by Schopfer and Jung ('37) and modified by Hamner ('43).

There was exceptional agreement on the thiamine values for vegetables from the two laboratories. This comparison is given in table 3, for the food as served.

Samples of vegetables were collected as follows: (1) frozen product before defrosting, or a sample of the raw product; (2) cooked product, immediately after cooking; (3) cooking water, immediately after vegetables cooked; and (4) steam table sample at 12 o'clock noon, when the serving ends.

In table 2 is recorded the total loss of vitamins from the vegetables prepared. The losses of vitamins in the vegetables during the cooking and holding processes are variable. Excluding the potato, the thiamine losses were 16 to 64%, with an average of 37; niacin losses 2 to 61%, average 27; riboflavin losses 22 to 45%, average 36; ascorbic acid losses 27 to 90%, average 65. While this research was being conducted, a trained home economist was working in the kitchen on the problem of the best methods to employ in cooking vegetables in mass feeding. Therefore, the amount of water used in cooking a given amount of vegetables, the cooking time, and the holding time after cooking had not been completely standardized.

It was not possible to estimate the exact losses in the different steps of preparation, but determinations for ascorbic acid indicate that of the total per cent lost, 3 to 65%, with an average of 21, was lost in the cooking water; 24 to 77, average 56%, destroyed in the cooking process; 0 to 67, average 22% destroyed on standing.

Table 3 was prepared to show what and how much of the various vitamins the customer actually got from a serving of the different vegetables as prepared in the cafeteria kitchen.

TABLE 2

Vitamin losses in cafeteria-prepared vegetables.

VEGETABLE	COOKING METHOD	AMOUNT Prepared	AMOUNT Water used	COOKING TIME	HOLDING TIME [1]	THIAMINE	RIBOFLAVIN	NIACIN	ASCORBIC ACID
		lbs.	*gal.*		*min.*	%	%	%	%
Peas, frozen	Boiled	10	½	27	38	25	38	41	68
Peas	Boiled	20	(?)	20	130	50	..	..	65
Lima beans, frozen	Boiled	60	9½	50	165	63	..	..	72
Lima beans, frozen	Boiled	20	1¼	25	130	37	34	22	44
Lima beans, frozen	Boiled	40	4½	40	50	43	35	45	27
Lima beans, frozen	Boiled	20	1½	33	50	35	33	35	77
Green beans, frozen	Boiled	20	2¼	45	115	55	..	..	..
Green beans, frozen	Boiled	40	4	25	120	43	4	30	..
Green beans, frozen	Boiled	20	1½	27	58	15	36	34	66
Green beans, frozen	Boiled	20	1½	29	68	33	15	34	74
Spinach, frozen	Boiled [2]	40	3	45	90	64	..	..	80
Spinach, frozen	Boiled	20	¼	23	17	22	22	..	65
Potatoes, raw	Steamed	85	..	60	65	2	10	10	90
Potatoes [3]	Steamed	300	..	40	30	7	2	2	51
Carrots, raw	Boiled	100	4	45	..	52	61	45	23

[1] Time between completion of cooking and the serving time (11: 30 A.M.)

[2] Spinach defrosted in several gallons cold water. Water drained off and spinach cooked, in fresh boiling water. There was 49% loss of thiamine and 52% loss of ascorbic acid by defrosting, before cooking.

[3] Potatoes (both samples) peeled day before used, covered with cold water, put in refrigerator overnight. Water drained before steaming — hence the large losses of ascorbic acid. Thiamine in several samples of potatoes tended to increase in value. Milk was added when potatoes were mashed.

TABLE 3

Vitamin content of a serving of cafeteria prepared vegetables.

VEGETABLE	SIZE PORTION	VITAMIN A	THIAMINE	RIBOFLAVIN	NIACIN	ASCORBIC ACID
	gm.	*I.U.*	*µg.*	*µg.*	*mg.*	*mg.*
Peas, frozen	85	496	220	80	1.45	3.06
Peas, frozen	85	567	150	..	...	3.40
Lima, beans, frozen	85	240	40	..	...	3.23
Lima beans, frozen	85	...	40	40	0.48	1.79
Lima beans, frozen	85	14	50	40	0.45	2.47
Lima beans, frozen	85	240	50	40	0.77	2.96
Green beans, frozen	85	42	50	80	0.21	3.74
Green beans, frozen	85	496	40	60	0.23	2.89
Green beans, frozen	85	467	60	60	0.37	2.55
Spinach, frozen	85	8216	30	..	...	3.49 [1]
Spinach, frozen	85	7450	60	..	0.32	14.53
Potatoes	112	0	80	60	0.32	1.68
Potatoes	112	0	100	80	0.40	3.80
Potatoes	112	0	100	40	0.59	4.80
Potatoes	112	0	100	80	0.40	4.70
Cole slaw	85	neg.	50	30	0.33	3.15
Carrots	85	...	30	20	0.13	4.08
Carrots	85	9605	30	30	0.16	3.49
Turnips	85	28	53	30	0.33	7.82

[1] See footnote, table 2, for method of defrosting.

DISCUSSION

Some problems in industrial feeding

Although menu planning and food selection are important, other factors which help to improve the nutritive value of the meals served are kitchen management and improved methods of food preparation. The observations mentioned here were made in only one cafeteria. This particular establishment prepared food for an office worker's dining room serving approximately 1400 people, as well as their own dining room serving 1000 to 1400 laborers.

In the present case operations were improved as much as possible while the study was in progress, and after it was completed, in order that the vitamin values might be conserved as much as possible. This involved decreasing the holding time between preparation and serving. Even with inexperi-

enced labor this was possible since meals were served according to a definite schedule.

In general, schemes that help speed service are: (1) posting menus in large print in strategic places so that the customer can make his own choice before he gets to the counter; (2) decreasing daily choice as much as possible; adequate choice can be extended over a period of time in the menus; (3) making the main dish choices as distinct from each other in appearance, flavor and cost as possible. When they are too much alike it causes confusion and indecision.

Decreasing choice aids in kitchen management. The preparation of fewer items simplifies the kitchen schedule, improves the work of untrained help and increases the efficiency in the use of limited equipment.

Decreased waste of food resources results from tested recipes which insure certain nutritive values per serving, such as protein and calories, and, as far as possible, minerals and vitamins.

SUMMARY

Preliminary studies were made in a large industrial cafeteria to determine the losses in vitamins in vegetables under actual operating conditions. The losses, in per cent, ranged as follows: thiamine 16 to 64; niacin 2 to 61; riboflavin 22 to 45; ascorbic acid 27 to 90. Changes were then made in the vegetable cookery by decreasing radically the time of cooking large quantities. The time of holding vegetables between cooking and service was also cut to a minimum. Texture, flavor and appearance were improved.

A comparison of the thiochrome and fungus assay methods for the determination of thiamine gave excellent results for analyses in vegetables.

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ADEQUACY OF THE INDUSTRIAL LUNCH AND THE USE OF BREWER'S YEAST AS A SUPPLEMENT

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(Received for publication March 22, 1943)

The purpose of the present study was to determine the adequacy of the vitamins in the noon meal consumed by a group of workers in the Brooklyn Navy Yard. After this was determined, brewer's yeast was introduced into certain cooked products to increase the water soluble vitamins in this noon meal.

The problems of feeding in a large plant such as the Navy Yard are numerous. The distance between the various cafeterias is great; the time for eating is short; the number of men is large; there are many problems in cookery. All contribute to the difficulty of supplying a third of a day's requirements in vitamins. One method of helping to overcome this difficulty is to increase the vitamins in the food by the addition of some natural product such as yeast.

Until relatively recent times man has been accustomed to consume yeast in various forms such as that found in beer. The beer now produced is very clear and quite free from yeast. Other uses are worthy of consideration. This problem has received our attention.

In preparation for the use of yeast in foodstuffs, several months were devoted to testing yeast in various forms and recipes. Yeast makes pleasing combinations with syrups or peanut butter. It can also be used in baked products such as breads, cookies and doughnuts. Considerable amounts of

yeast can be incorporated into meat loaves, stews, cheese dishes, baked beans and meat balls. Yeast cannot be used in corn bread, biscuits or in other mildly flavored recipes.

Yeast has been converted into both tablets and pellets. The most satisfactory combination for these consists of equal parts of dry skim milk and dry brewer's yeast. The cost of manufacturing pellets is only a small fraction of that for tablets.

To a limited extent dry yeast has been employed in human foodstuffs for many years, especially since its value has been appreciated in treating pellagra. Carr ('41) has published recipes for the use of yeast in home cookery. Johnson and others ('42) found yeast useful in providing a source of thiamine in their studies of exercise. For many years yeast has been employed in various studies at Cornell (Savage and McCay, '42). In the course of this work its merit has been appreciated not only because it is an inexpensive natural source of recognized vitamins but also because many experiments indicate that it contains essential factors that have never been isolated and recognized.

EXPERIMENTAL

The studies reported here were made in the kitchens and cafeteria of the Brooklyn Navy Yard. Complete meals as well as the various items offered in the menu were selected in such a manner as to get representative samples. After weighing, these were put through a Waring Blendor. Two aliquots were then packed in dry ice. One was shipped to Fordham University and the other to Cornell. Thiamine was determined at both Fordham and Cornell; niacin and riboflavin were determined at Cornell, by the methods described elsewhere (Heller et al., '43).

Two types of meals were studied, namely cafeteria meals and a lunch [1] prepared and then packaged in individual paper containers, kept warm in an oven until lunch time and then served on paper trays, from outdoor stands. They usually consist of 7 to 10 ounces of soup, a main dish (such as a stew,

[1] Mealpak.

beans and frankfurters, macaroni and cheese, etc.), two slices of white enriched bread, a pudding or jello dessert, occasionally a small vegetable salad, and a choice of either milk or coffee.

The lunches are employed to help speed the distribution of food and to overcome the disadvantage of the distance between groups of workers.

The cafeteria meals usually consist of a roast meat, meat-vegetable dish, or meat alternate such as macaroni creole, etc., mashed potatoes, one other vegetable (such as green lima beans, green peas, green beans, carrots, spinach or turnips), two slices of rye, whole wheat or enriched white bread, one pat of butter, choice of dessert (usually pie), and choice of coffee, tea or milk.

All the men eating in the cafeteria do not select such a hearty meal as described above. A large number of them do, but many bring their sandwiches from home and supplement them with coffee, and dessert from the cafeterias or stands. Many of the naval personnel eating in the cafeteria select milk as a beverage. About one-fourth to one-third of the workers select milk.

The results of the thiamine determinations are shown in table 1. The fungus assay method applied for thiamine analyses in whole meals gives higher values than the thiochrome technique. This contrasts with the excellent results for vegetables by the two methods, reported by Heller and co-workers ('43). The error due to meat must be much larger than that indicated for the whole meals, since the accuracy on vegetables would reduce the discrepancy in the meal. In the following discussions, the fungus assay values have been used.

The results of all the vitamin determinations are summarized in table 2.

DISCUSSION

The noonday meal fails to furnish a third of the day's vitamin needs if the accepted standards are correct. The morning meal cannot be expected to furnish a third unless eating habits are much improved. Breakfast can be improved by using better

bread for toast, more citrus fruit, and doughnuts made with liberal additions of such products as soy bean flour and brewer's yeast.

After these initial studies indicating the level of vitamins ingested in the noonday meal, dried brewer's yeast was added to certain of the cooked products to see if it could be incorpo-

TABLE 1

Thiamine content of lunches[1] and cafeteria meals. Comparison of values from thiochrome and fungus assay methods.

CAFETERIA MEAL NO.	FUNGUS ASSAY (CORNELL)	THIOCHROME (FORDHAM)	LUNCH NO.	FUNGUS ASSAY (CORNELL)	THIOCHROME (FORDHAM)
	μg./gm. fresh wt.	*μg./gm. fresh wt.*		*μg./gm. fresh wt.*	*μg./gm. fresh wt.*
I	...	1.55	I	0.22	...
II	0.79	0.46	II	0.28	...
IV	0.66	0.47	III	0.58	...
V	0.68	0.45	IV	0.19	...
VI	0.94	0.62	V	0.34	...
VII	0.59	0.44	VI	0.46	0.31
VIII	...	1.26	VII	0.78	0.45
X	1.46	1.19	VIII	0.66	...
XI	0.61	0.49	IX	0.57	...
XII	0.73	0.54	X	0.84	0.52
XII	0.74	0.58	XI	0.90	0.62
XIII	...	2.89	XV	1.46	0.97
			XVI	0.41	0.27
			XVII	0.48	0.35
			XVIII	0.83	0.66
			XIX	0.71	0.53

[1] Mealpaks.

TABLE 2

Contribution of the noon luncheon to the day's needs.

TYPE OF MEAL	NO. OF CASES	THIAMINE		RIBOFLAVIN		NIACIN	
		Mean (range)	Fraction of daily need[1]	Mean (range)	Fraction of daily need[1]	Mean (range)	Fraction of daily need[1]
		mg.	%	*mg.*	%	*mg.*	%
Lunch	16	0.54 (0.17–1.20)	23	0.59 (0.30–1.65)	18	3.37 (1.20–5.99)	15
Cafeteria	9	0.62 (0.39–1.06)	27	0.57 (0.30–1.65)	17	6.05 (2.49–9.65)	26

[1] Assumed dietary allowance for the working man: 2.3 mg. thiamine; 3.3 mg. riboflavin; 23.0 mg. niacin.

rated at a reasonable level without injury to the flavor and appearance of the food. Considerable initial effort was given to standardizing the recipes.[2] Yeast was added to the following: stuffed green peppers, meat loaf, meat balls, pork goulash, lima bean creole, veal or lamb curry, salmon salad sandwich spread, cheese sandwich spread and bean-frankfurter spread. It was also added to the beef goulash as well as to the beef-kidney stew by the chef.

TABLE 3

Comparison of thiamine content of main dishes with and without dry brewer's yeast, by thiochrome and fungus assay methods.

FOOD	SIZE OF PORTION	FUNGUS ASSAY (FRESH WT.)	THIOCHROME METHOD (FRESH. WT.)
	gm.	*μg./gm.*	*μg./gm.*
Pork goulash	196	2.6	3.9
Pork goulash + yeast		14.7	10.7
Curry of veal	196	0.35	0.35
Curry of veal + yeast		7.5	7.9
Meat loaf	84	1.1	1.1
Meat loaf +yeast			19.8
Beef and kidney stew	196	0.81	0.48
Beef and kidney stew + yeast			4.4
Beef goulash	196	0.72	0.36
Beef goulash + yeast		3.73	3.15
Lima bean creole	196	1.59	1.09
Lima bean creole + yeast			4.23
Stuffed green peppers	112	1.2	...
Stuffed green peppers + yeast		14.3	15.09

The yeast added was a product rich in thiamine,[3] containing 723 μg. of the vitamins per gram. The increase in the thiamine obtained by adding brewer's yeast to some main dishes is shown in table 3. This table also gives the average helping of the different products.

Ordinary brewer's yeast contains the following vitamins per gram: thiamine, 150 μg.; riboflavin 50 μg.; and niacin 600

[2] Standardized recipes were developed by Marcia Wolf, Nutritionist for the Canteen Food Service.

[3] Supplied by the Yeast Products Corporation.

μg. With the very conservative additions of yeast that were used at the level of these initial attempts, the average increase in thiamine if ordinary yeast were used would be 0.55 mg. (range 0.20 to 0.90 mg.); corresponding figures for riboflavin and niacin would be 0.18 (0.07 to 0.30 mg.) and 2.18 (0.80 to 3.60 mg.) respectively.

Dry brewer's yeast could have been used at a higher level, especially if yeast with a mild flavor had been employed. The values given here probably represent a low starting level for large scale cookery.

Attempts were made to determine vitamin C and carotene contents of the composite samples of mixed foods from these meals. In both cases so many difficulties were encountered that the results are regarded as valueless. Carotene values in many cases tended to be higher in the cooked meal than in similar ingredients before cooking. In the case of vitamin C, also, the values for the meal were too high from calculations based upon the individual items in a given meal.

SUMMARY

The meals served workers in one cafeteria in the Brooklyn Navy Yard have been sampled for the determination of riboflavin, niacin and thiamine. This noon meal usually furnishes at most only one-fourth of the day's requirements for these vitamins. Dry brewer's yeast has provided a satisfactory method of supplementing the supply of these vitamins. This yeast was usually incorporated in the meat dishes at conservative levels, so that the taste was not detected. Since yeast is rich in protein of good quality it also affords a good source of this dietary factor.

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FACTORS INFLUENCING THE BIOASSAY OF VITAMIN E[1]

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(Received for publication March 8, 1943)

INTRODUCTION

The establishment of a standard method of vitamin E assay has been in progress since 1927, when Evans and Burr made observations of the fetal resorptions in female rats deprived of vitamin E. Bacharach ('38) defined the mean fertility dose as that dose showing a response of a live litter in 50% of the implanted animals. Bacharach and Allchorne ('38) define a litter as consisting of one or more dead or live young. Some workers have been using the mean fertility dose as the dose denoting vitamin E "activity". Palmer ('37) expresses the results in per cent efficiency, not only of the live litter, but also of the total (live plus dead) litter, total implantation and live implantation. These efficiencies are defined, respectively, as the percentage of rats in a group producing live litters, producing live and dead litters, the percentage of total implantations resulting in live and dead young and those resulting in live young. There are certain advantages in using the implantation efficiencies in addition to the litter efficiencies which make this method preferable. The autopsy is conducted after parturition in both of these methods. Palmer ('37) distinguishes between live, dead and resorbed fetuses. Any response having a live implantation efficiency of 10% or more is considered a positive response. Mason ('39, '42) employs the formula: $\frac{W+N}{5}$ = "uterine index", where W represents the

[1] Paper no. 47, Journal Series, General Mills, Inc., Chemical Research Department.

weight of the uterine contents and N the number of viable young when two or more are present. Uterine index values of less than 0.35, 0.35 to 0.99 and 1.0 or greater are classed, respectively, as negative, subminimal and positive responses. Mason performs the autopsy on the sixteenth day of pregnancy.

Evans and Burr ('27 b) reported that 22% lard was usually sufficient to inactivate the vitamin E in wheat germ even when the latter was incorporated in the diet at a level of 2%. Macomber ('33) reported that rats fed a diet containing 20% lard and supplements of 0.5 gm. or more of wheat germ became sterile. The animals reproduced when lettuce was fed separately even though the diet contained 20% lard. Rancid fats have frequently been held responsible for the destruction of vitamin E as shown in animal studies. It was reported by Weber, Irwin and Steenbock ('39) that there was no destruction of vitamin E when the latter and rancid fats were fed separately, but that three times as much vitamin E was required when the diet was composed of rancid fat rather than fresh fat. The present study was made to investigate some of the factors influencing the bioassay of vitamin E.

EXPERIMENTAL

The method of assay is similar to that described by Palmer ('37) except for a number of modifications made by this laboratory. Ten day old females and their dams are transferred to Palmer's ('37) vitamin E-deficient diet.[2] At 70 days of age the young females are placed in individual cages and daily vaginal smears are made, using the procedure described by Long and Evans ('22). Evans and Burr ('27 a), Palmer ('37) and Bacharach and Allchorne ('38) report a high rate of pseudopregnancies, sometimes as high as 20%. We have experienced a very low rate, seldom exceeding 2%. We attribute this to our close observation of the males and the discarding of all those whose breeding performances are questionable. We have noted that pseudopregnancies are most prevalent when the males are used too frequently. Any female not mat-

[2] The vitamin E-deficient diet is kept under refrigeration between feedings.

ing within 2 weeks following the depletion period is discarded. All the tests are conducted with virgin rats, because our experience has been that the vitamin E requirement for production of the second litter is higher than it is for the first litter. Initial doses of the test material are fed on the first day of pregnancy. Dry samples are either incorporated in the vitamin E-deficient diet or fed per se. Supplements consisting of oil or ethyl laurate solutions are fed orally in doses of 0.10 ml. or less by means of a 0.25 ml. calibrated syringe. Supplements are fed at a rate of three doses of 0.1 ml. per day until the total dosage has been fed. Supplements in excess of 3.0 ml. are fed in doses of 0.10 ml. during the first 10 days of gestation, so that one-tenth of the total supplement is fed each day. The autopsy of the test animal is made on the twenty-first day of gestation from 12 to 24 hours prior to parturition. The autopsy consists of an examination of the reproductive organs and their contents to determine the number of live young, dead young, resorption sites and implantation sites.

The conclusions drawn in our bioassays are based on both the live litter and the live implantation efficiencies. A 50% live litter efficiency and a 20% live implantation efficiency are taken as the lowest average response of a group of seven rats indicating vitamin E activity.

Implantation rate

The number of implantation sites of 2528 vitamin E assay rats [3] were tabulated (table 1). Their responses may be divided into three groups: those of less than seven implantations; those of seven to fourteen (inclusive) implantations; and those of more than fourteen implantations. If the above-mentioned first and third groups are discarded, it will mean a loss of 3.4% of the rats. Compared with the percentage of animals discarded in bioassays of some of the other vitamins, 3.4% is a relatively low percentage of discards. It has been learned that responses falling within these two groups have a

[3] These rats were used in assaying a large variety of samples, such as wheat germ oil, wheat germ, etc.

great effect on the average response of the assay group. The elimination of these responses would decrease the risks of interpreting, for example, a response of two live young from a rat having a total of two implantations, as equivalent to a response of eleven live young from a total of eleven implantations. The same principle may also be applied to rats having responses of more than fourteen implantations. The questionable interpretation of these responses accounts for much of the error in some vitamin E assays and the failure to consider the implantation efficiency also increases the percentage of error.

TABLE 1

Showing the per cent distribution of implantation sites of 2528 vitamin E assay rats.

NO. OF IMPLANTATION SITES	NO. OF RATS	PER CENT OF TOTAL	NO. OF IMPLANTATION SITES	NO. OF RATS	PER CENT OF TOTAL
1	3	0.12	10	526	20.7
2	7	0.27	11	605	23.9
3	7	0.27	12	431	17.4
4	7	0.27	13	247	9.7
5	13	0.51	14	90	3.6
6	9	0.35	15	29	1.1
7	57	2.3	16	6	0.23
8	149	5.8	17	5	0.20
9	336	13.2	18	1	0.04

Dosing

Mason ('39) found that the procedure employed in the administration of vitamin E has a pronounced effect on the efficiency of the absorption or the utilization of the vitamin. Dosing prior to mating rather than dosing during pregnancy results in lower reproduction efficiency. Mason also found that single doses fed on the eighth day of pregnancy yielded the most satisfactory results. However, it is difficult to feed the total dosage of low potency samples in a single day, and furthermore it may influence the consumption of the basal diet. A group of tests (table 2) were run with whole wheat to determine a satisfactory procedure for assaying samples of a low vitamin E potency. The sample of whole wheat used in these

tests was previously found to be active at a level of 45 gm., when fed at a rate of 4.5 gm. per day during the first 10 days of pregnancy. The lessened effectiveness of dosing prior to mating is indicated by groups 2 and 3 and also by groups 4 and 5 (table 2).

A study was made of the effects of cod liver oil (U.S.P.), lard (commercially packaged lard), tapioca dextrin (autoclaved tapioca), enriched patent flour [4] and fat-free enriched patent flour [5] on the vitamin E activity of high- and low-potency wheat germ oils (table 3). These tests were all conducted by means of the experimental procedure previously

TABLE 2

Showing the effect on vitamin E assays when supplements of ground whole wheat are fed before and after mating.

GROUP NO.	RATS NO. OF	GRAMS OF WHEAT FED PER DAY	DAYS OF DOSING		LIVE LITTER EFFICIENCY	LIVE IMPLANTATION EFFICIENCY
			Before mating	After mating		
1	3	0	..	..	0	0
2	7	6	10	..	0	0
3	4	6	..	10	75	74
4	5	3	10	10	80	51
5	5	3	..	20	100	98

described. The lard and cod liver oil were withheld from the basal diet as indicated in table 3. The supplements of tapioca dextrin, enriched patent flour, and fat-free enriched patent flour were incorporated with the basal diet as indicated. The wheat germ oils and the fat extract of enriched patent flour were fed by means of a syringe. All the supplements were fed during the first 10 days of pregnancy at the rate of one-tenth of the total dosage per day. The regular basal diet was fed ad libitum to all rats from the tenth day of pregnancy until the time of autopsy.

In table 3, groups 2 and 3 correspond to the regular vitamin E assay of wheat germ oil with the exception of the longer

[4] The thiamine, niacin and iron contents per pound of enriched patent flour as specified in Federal Register 6, 2580, 1941 are 1.66 mg. to 2.5 mg., 6.0 mg. to 24 mg. and 0.6 mg. to 24 mg., respectively.

[5] The fat content of the enriched patent flour was 1.0%.

dosing period. Enriched patent flour showed no vitamin E activity when it was fed at the 60 gm. level, however groups 10 and 12 indicate that 30 gm. of the flour exert as great an effect on littering as 150 mg. of wheat germ oil. The influential effect of patent flour is appreciable as shown also by a comparison of the responses of groups 3 and 6. It is shown by the results of groups 3 and 19 and 2 and 15 that the presence of lard and cod liver oil in the basal diet interferes with the ab-

TABLE 3

Showing the effects of tapioca, enriched patent flour, fat-extracted patent flour, fat extract of enriched flour, lard and cod liver on the vitamin E potency of high- and low-potency wheat germ oils.

GROUP NUMBER	NUMBER OF RATS	DIETARY FACTORS TESTED								LIVE LITTER EFFICIENCY	LIVE IMPLANTATION EFFICIENCY
				Amounts fed as supplements							
		Lard: in basal diet	Cod Liver Oil: in basal diet	Tapioca	Enriched patent flour	Fat-extracted enriched patent flour	Fat extract of enriched patent flour	High-potency wheat germ oil	Low-potency wheat germ oil		
		%	%	gm.	gm.	gm.	gm.	mg.	mg.	%	%
1	8	22	2	0	0	0	0	0	0	0	0
2	7	22	2	0	0	0	0	0	500	14	13
3	6	22	2	0	0	0	0	500	0	67	40
4	6	22	2	0	60	0	0	0	0	0	0
5	7	22	2	0	60	0	0	0	500	57	28
6	6	22	2	0	60	0	0	500	0	100	80
7	7	22	2	60	0	0	0	0	500	57	28
8	7	22	2	0	0	59.4	0	0	500	57	31
9	7	22	2	0	0	0	0.6	0	500	43	7
10	6	22	2	0	60	0	0	350	0	100	51
11	6	22	2	0	60	0	0	200	0	33	5
12	6	22	2	0	30	0	0	500	0	83	51
13	6	22	2	0	30	0	0	350	0	67	17
14	6	22	2	0	30	0	0	200	0	0	0
15	7	0	0	0	0	0	0	0	500	71	48
16	7	11	1	0	0	0	0	0	500	71	33
17	7	0	2	0	0	0	0	500	0	71	39
18	7	22	0	0	0	0	0	500	0	71	24
19	7	0	0	0	0	0	0	500	0	100	81

sorption or utilization of vitamin E from wheat germ oil. The absence of either the lard or the cod liver oil does not improve the utilization of vitamin E; however, the absence of both or a 50% reduction of both in the basal diet improves the reproduction efficiency of the assay rats. It may be noted that the vitamin E-deficient diet consists of 35% tapioca dextrin and that a 60 gm. supplement of this dextrin exerts an effect on vitamin E utilization nearly similar to those of 60 gm. supplements of enriched patent flour and fat-extracted patent flour. It may likewise be noted (groups 6, 19 and 3) that 60 gm. of patent flour and the fat-free basal diet yielded results of 100% live litter efficiency and 80% and 81% live implantation efficiency, respectively, as compared with 67% live litter efficiency and 40% live implantation efficiency when the regular assay procedure was used. The fat intake is lowered from 75% to 80% when a 60 gm. supplement of patent flour or tapioca dextrin is fed over the 10 day period. Therefore, it appears that the increased vitamin E activity, as a result of feeding supplements of dextrin or patent flour, is due to the lowering of the intake of lard and cod liver oil.

SUMMARY

The methodology of the vitamin E bioassay has been studied and it has been noted that the assay results are improved by discarding rats having less than seven and more than fourteen implantations.

The lessened effectiveness of dosing prior to mating was demonstrated.

Autopsy of the assay rats prior to parturition eliminates possible errors due to infanticide.

The high rate of pseudopregnancies (10 to 20%) reported by other workers was not experienced.

The vitamin E activity of an assay sample is influenced by the presence of 22% lard and 2% cod liver oil in the basal diet during the dosing period. The use of a fat-free basal diet during the dosing period results in much higher live litter and live implantation efficiencies.

ACKNOWLEDGMENT

The author wishes to acknowledge the assistance of Miss Claire Frederick and Dr. John S. Andrews.

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THE PERFORMANCE OF NORMAL YOUNG MEN ON CONTROLLED THIAMINE INTAKES[1]

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TWO FIGURES

(Received for publication March 3, 1943)

For our purposes we define the thiamine requirement of man to be the amount needed to allow maximal and most efficient performance of the body. Practical application, however, requires further definition. What functions and characteristics of the body should we measure?

Outstanding characteristics of definite thiamine deficiency are weakness, fatigue, neuro-muscular incoordination, anorexia and disturbances of carbohydrate metabolism. With the exception of anorexia these characteristics are capable of quantitative evaluation under controlled conditions. Such measurements under rigidly standardized performance test conditions should allow estimation of the level at which thiamine restriction does, in fact, affect the functional capacity and efficiency of the organism. This approach was applied in the studies reported here. In addition urinary excretion and subjective reports were examined.

[1] The expenses of the work described in this paper were defrayed in part by funds provided under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Minnesota. Important financial assistance was also provided by the U. S. Cane Sugar Refiners' Association, by the Corn Industries Research Foundation, by the National Confectioners' Association, by Swift and Company, and by the Nutrition Foundation. Merck and Company, Inc., supplied both financial assistance and the pure vitamins. Valuable aid was given by the Work Projects Administration under the University of Minnesota project no. 8760, subproject 380.

We have emphasized the following: (1) ability to do brief severe work of the type that is largely anaerobic; (2) ability to do moderately prolonged work of a severity close to the maximum at which a relatively steady state can be maintained; (3) ability to carry out various tasks of psychomotor function involving speed and coordination; and (4) ability to maintain normal carbohydrate metabolism, including its intermediary details.

SUBJECTS

All subjects were men students between the ages of 18 and 30 years, free from signs and history of significant abnormality. None had peculiar habits of diet or activity. They were selected for personal reliability and were under daily observation but were not confined except at mealtimes and for 1 day each week. All were physically active but none was engaged in regular team sports. Outside eating was strictly forbidden except on rare occasions by special arrangement. A very small number of lapses occurred and were reported; these proved to be unimportant.

DIET

All meals were prepared in the diet kitchen of the Laboratory. Care was taken to make the food as attractive and palatable as possible. Every portion was weighed and an extra identical meal was put aside for analysis at each meal time. In the first two series these meals were ground, quick-dried in a drier at 40–45° C., and then stored at –25° C. until analyzed. This procedure proved to allow significant losses of thiamine so thereafter the meals were ground and either analyzed immediately or quick-frozen and stored at –25° C. for analysis. The thiamine content of the diet for each day was also calculated from tables assembled from the literature.

The basal diet contained 340 to 380 gm. of carbohydrate, 100 to 120 gm. of protein, 110 to 130 gm. of fat and furnished 3,000 Cal. per day. Slight deviations, $\pm$ 200 Cal., sufficed to maintain body weight almost exactly constant in all subjects.

Each subject received a daily supplement of 2 mg. riboflavin, 25 mg. nicotinic acid amide and 50 mg. of ascorbic acid.

In series I and II half of the subjects received an additional 1 mg. of thiamine daily while the other subjects received placebos. The two groups were reversed in the second half of these experimental periods. At the end of experimental series III and IV each subject was given 4.11 mg. thiamine chloride by mouth and the excretion of thiamine was measured in the urine for the succeeding 24 hours.

ANALYTICAL METHODS

The 24 hour urine sample was collected in a glass bottle containing 5 cc. of glacial acetic acid and 5 cc. of toluol. The samples were stored at 5° C. for not more than 24 hours after collection. Thiamine was estimated by the thiochrome method of Hennessy and Cerecedo ('39) as modified by the Research Corporation Committee for the determination of thiamine in cereal products (privately circulated, '41). The same thiamine method was applied to the foods. With both foods and urine, tests of recovery of added thiamine were made.

Venous blood, drawn with a minimum of stasis, was analyzed for lactate by the method of Friedemann and Cotonio as modified by Edwards ('38). Pyruvate was estimated in the blood by the method of Lu ('39) as modified by Friedemann and Haugen ('43).

Blood sugar was measured by the method of Folin and Wu. Hemoglobin was measured as oxyhemoglobin in the photoelectric colorimeter. Blood ketones were estimated in series I by the method of Barnes and Wick ('39).

PSYCHOMOTOR TEST METHODS

The following tests were used as a "battery" covering: (1) manual speed and coordination, (2) motor control, and (3) velocity of small hand movements. They were designed so they could be carried out during actual work on the treadmill. The three tests were given in consecutive order of manual speed, motor control and tapping, with a rest of 30 seconds

between each test. The duration of the manual speed and motor control tests was 60 seconds each; the tapping test lasted 30 seconds in which the rates were measured for each 10 seconds.

Manual speed (Brozek, '43) was tested with an open pipe 30 cm. long, and 1.3 cm. internal diameter, which was held vertically by the left hand. The task was to place a steel ball, 1.3 cm. in diameter, in the top of the pipe, catch it at the bottom and repeat the cycle as fast as possible. A mechanical counter recorded the number of times the ball passed through the pipe. A cloth bag attached by a wire hoop at the bottom of the pipe served to stop the balls not caught.

Motor control was tested by tracing a winding path 1.1 cm. wide and 0.6 cm. deep with a stylus 0.5 cm. in diameter. The path was 500 cm. long arranged in a field 48 by 35 cm., and provided a variety of movement patterns (short and long, angular and circular, proceeding right and left). The number of contacts and the total duration of contacts between the stylus and the side walls of the path were recorded electrically and served as performance criteria.

Velocity of small hand movements was tested by tapping, as rapidly as possible, 2 brass plates 4.8 cm. in diameter and placed 1.7 cm. apart. The plates were separated by a wooden barrier 1 cm. high and were tapped alternately (to eliminate tremor tapping). The number of taps was recorded electrically in the first and the third 10-second intervals of a 30-second work period.

EXPERIMENTAL PROGRAM

There were four series of experiments each lasting 10 to 12 weeks exclusive of the preliminary period of training and standardization. There were four subjects in each series; three of the subjects took part in three series each so that there was a total of ten subjects and extensive control data were accumulated on three of the men. The average thiamine intakes in the four series (I, II, III, IV) were, respectively, 0.63, 0.53, 0.33 and 0.23 mg. per 1,000 total Calories.

During the first half of series I and II half of the subjects received a daily supplement of 1 mg. of thiamine while the other men received placebos. During the second half of these series the order was reversed so that the men formerly on placebos now received the thiamine supplement and vice versa. None of the men knew either of these arrangements or of the contents of the other tablets (ascorbic acid, riboflavin and niacin) which all men received daily in all experiments.

In all four series the general arrangement of tests was the same. Once a week each man was studied, in the basal post-absorptive state, in a half-day series of tests in a constant environment of 78° F. and about 50% relative saturation. The subject was dressed in shoes, shorts and undervest and returned to bed for 20 to 30 minutes before a blood sample was taken. The cardiotachometer was then attached and the standing heart rate was recorded for 3 minutes before these psychomotor tests were started. At the end of these experiments, work was started on the motor-driven treadmill. The first exercise test consisted of walking at a rate of 3.85 miles per hour at a 12% rate of climb. This means accomplishment of external work at a rate of 870 kg. meters per minute by a man weighing 70 kg., and required an oxygen usage of about 2730 cc. per minute and an expenditure of about 820 calories per hour. In series I this work period was 90 minutes; the work period was 60 minutes in series II, III and IV. The heart rate was recorded during work and during recovery except while psychomotor tests were in progress. The battery of psychomotor tests was run through once in the first 15 minutes of work, again in the last 15 minutes of work and finally in recovery starting 5 minutes after the end of work.

In series II, III, and IV this first exercise test was followed, after 10 minutes for partial recovery, by 2 minutes of running at 7 miles per hour at a 10% rate of climb (1316 kg. meters per minute for a 70 kg. man).

Every second week blood samples were drawn at 3 and at 10 minute intervals after walking and at 5 and 15 minutes after running. Twenty-five minutes after running 100 gm. of glu-

cose were ingested and further blood samples were then taken at intervals of 30, 60, 90 and 120 minutes while the subject rested quietly.

Roentgenograms of the heart and electrocardiograms were made on each subject at the start and at the end of the restricted thiamine periods.

Thiamine was measured in the 24 hour urine in all except series I. At the end of series III and IV a thiamine saturation test was made on the day following the last 24 hour urine collection.

RESULTS

The data collected are so extensive that they can only be presented as averages and examples.

General observations and subjective reports

Apart from the initial adjustments to the rigid regime and some slight training effects, both personal characteristics and performance appeared to be remarkably constant in all subjects in all four series. The close daily contacts between subjects and observers gave ample opportunity to observe changes in behavior and apparent ability to carry out the several tests. Muscle hyperesthesia or calf tenderness did not occur in spite of the weekly periods of severe walking and running exercise. No obvious changes were seen. We were fortunate that neither illness nor emotional crises occurred at any time.

The subjects were questioned for subjective reports from time to time. Care was taken to avoid suggestive questions. The normal variations in vigor and sense of well-being were recorded, these oscillations being neither unusually frequent nor intense. There was occasional worry over college examinations, a few mild states of the "blues" and a complete absence of any trend or consistency in these subjective states. Certainly all general observations and subjective reports would suggest that it was a matter of complete indifference whether the men received 0.23 or 0.96 mg. of thiamine per 1,000 Calories.

Heart sizes and electrocardiograms

Heart sizes were measured in systole and diastole by the roentgenkymographic method (Keys et al., '40). No changes beyond the error of the method occurred at any time in any subject. The electrocardiograms (Leads I, II, and III) were likewise entirely negative. The points examined were: (1) Axis deviation, (2) P-Q interval, (3) Q-S interval, (4) voltage of R, (5) voltage of T, (6) regularity.

Heart rates

Table 1 shows that the heart rates while standing, during work and in recovery were unaffected by thiamine intakes of 0.53 and of 0.96 mg. per 1,000 Cal. Table 2 shows that sub-

TABLE 1

Heart rates in rest (standing), in the last minute of 90 (series I) or 60 (series II) minutes of severe work, and for the second 60 seconds of recovery. Averages, in beats per minute, for all subjects for all tests in the periods on the designated thiamine intakes; these last are in milligram per 1000 Cal.

PERIOD	THIAMINE			
	Series I		Series II	
	0.63	0.96	0.53	0.86
Standing rest	93	96	89	88
Last minute work	152	153	151	158
Second minute recovery	114	116	127	133

TABLE 2

Heart rates in rest (standing), in the last minute of 60 minutes of severe work and for the second minute of recovery. Averages, in beats per minute, for all subjects for the weeks after the start of thiamine restriction. Series III — 0.33 mg. per 1000 Cal.; series IV — 0.23 mg. per 1000 Cal.

PERIOD	WEEK					
	1	2	3	5	7	9
Series III						
Rest	90	...	95	91	99	91
Work	152	...	155	151	144	144
Recovery	130	...	126	130	126	118
Series IV						
Rest	89	86	...	95	88	97
Work	152	147	...	144	150	149
Recovery	126	121	...	119	124	128

sistence on thiamine intakes of 0.33 and of 0.23 mg. per 1,000 Cal. produces no progressive trend in the heart rates in either rest, severe work or in recovery.

In series I and II the blood lactate and pyruvate values were substantially identical at all times in the groups on the "high" as compared with the "low" thiamine intakes and this was true after work as well as in rest. Table 3 shows the averages for series III and series IV which indicate that the

TABLE 3

Lactate and pyruvate concentrations, in milligrams per 100 cc. blood, in series III and IV during subsistence on 0.33 and 0.23 mg. thiamine per 1000 Cal., respectively. Values in rest (Rest), *at 10 minutes after 60 minutes of work* (Work 2), *at 5 minutes* (Work 3) *and at 15 minutes* (Work 4) *after 2 minutes of extreme work, and at 60 minutes after ingestion of 100 gm. of glucose* (Tol. 2). *Values tabulated are averages for all subjects for the weeks after start of the thiamine restriction. L = lactate, P = pyruvate.*

SERIES AND TIME	1 WEEK		2 WEEKS		3 WEEKS		5 WEEKS		7 WEEKS		9 WEEKS	
	L	P	L	P	L	P	L	P	L	P	L	P
Series III												
Rest	15.6	2.02	...	...	16.4	1.91	14.9	1.98	18.8	2.04	13.6	1.60
Work 2	11.1	...	...	...	14.2	...	11.1	...	10.9	...	10.4	...
Work 3	58.2	3.50	...	...	62.0	3.35	63.7	3.30	56.2	3.18	43.9	3.18
Work 4	45.4	2.97	...	...	54.9	3.29	43.1	3.03	52.1	2.79	39.4	2.79
Series IV												
Rest	...	...	13.6	1.54	15.1	1.56	14.9	1.68	13.9	1.83	13.6	1.59
Work 2	...	...	15.8	...	19.6	...	14.5	...	20.7	...	19.0	...
Work 3	...	...	51.7	2.98	55.5	2.78	44.4	3.12	58.7	2.87	52.1	3.07
Work 4	...	...	45.6	2.81	51.2	2.67	44.7	3.03	64.6	2.87	46.8	2.84
Tol. 2	...	...	9.7	1.43	12.1	1.26	13.4	1.50	15.0	1.55	13.8	1.35

blood levels of these metabolites are unaffected in any consistent manner by thiamine intakes of 0.33 and of 0.23 mg. per 1,000 Cal. Further comparison may be made with the four subjects in series II who received from 0.53 to 0.86 mg. thiamine per 1,000 Cal. At the end of 10 weeks these "controls" averaged 1.79 mg. pyruvate per 100 cc. of blood in rest, 3.27 mg. for "Work 3," and 3.15 mg. for "Work 4" ("Work 2" blood was not drawn in the earlier series). It is notable that there was actually a fall in the pyruvate following glucose adminis-

tration (cf. Williams et al., '43). It is evident that in these young men the smallest thiamine intake used— 0.23 mg. per 1,000 Cal. — was sufficient to allow intermediary carbohydrate metabolism to proceed independently of the thiamine intake.

Blood sugar

Tables 4 and 5 give the average blood sugar values for the four series in rest, after work, and in glucose tolerance tests. From table 4 it appears that thiamine intakes higher than 0.63 or 0.53 mg. per 1,000 Cal. may be associated with slightly higher blood sugar levels at all these times. The significance

TABLE 4

Glucose concentrations, in milligrams per 100 cc. of blood, in rest (Rest), *at 3 minutes* (Work 1) *and at 10 minutes* (Work 2) *after 60 or 90 minutes walking, at 5 minutes* (Work 3) *and at 15 minutes* (Work 4) *after 2 minutes of extreme exertion and at 30, 60, 90 and 120 minutes after ingestion of 100 gm. of glucose* (Tol. 1, Tol. 2, Tol. 3, *and* Tol. 4, *respectively).*

THIAMINE INTAKE	REST	WORK 1	WORK 2	WORK 3	WORK 4	TOL. 1	TOL. 2	TOL. 3	TOL. 4
Series I, 0.63	77.8	74.2	76.8	...	...	135.5	154.2	134.9	119.7
Series I, 0.96	80.8	77.1	80.1	...	...	139.8	157.7	138.1	121.1
Series II, 0.53	79.2	79.1	80.9	81.7	78.2	140.7	145.6	123.9	....
Series II, 0.86	84.9	83.9	85.6	85.4	80.3	149.0	145.7	129.6	....

of this is uncertain though the relation is consistent and is not due to methodological error. However, maintenance at thiamine intakes of 0.33 or 0.23 mg. per 1,000 Cal. does not result in any tendency for the blood sugar levels or their regulation to change in any consistent fashion.

Psychomotor tests

There were steady but small increases from week to week in all the psychomotor test scores under all conditions. Presentation of the voluminous data from the psychomotor tests is hardly justified in view of the fact that statistical analysis of the scores in all three tests before, during, and after work showed only the same slight but steady improvement from

week to week, regardless of the thiamine intake. This was true for each of the test subjects as well as for the averages for all. The control data indicate that the rate of this gradual improvement was normal in the men on the restricted thiamine intake. Illustrative results for motor control are given in figure 1.

The psychomotor tests used here are as sensitive as any of which we have knowledge. It must be concluded then that re-

TABLE 5

Glucose concentrations, in milligrams per 100 cc. of blood, in series III and IV during subsistence on 0.33 and 0.23 mg. thiamine, respectively. Values in rest (Rest), *at 3 minutes* (Work 1) *and at 10 minutes* (Work 2) *after 60 minutes of work, at 5 minutes* (Work 3) *and at 15 minutes* (Work 4) *after 2 minutes of extreme work, and at 30, 60 and 90 minutes after ingestion of 100 gm. of glucose* (Tol. 1, Tol. 2, *and* Tol. 3, *respectively). Values tabulated are averages for all subjects for the weeks after start of the thiamine restriction.*

PERIOD	REST	WORK 1	WORK 2	WORK 3	WORK 4	TOL. 1	TOL. 2	TOL. 3
Series III								
1 week	86.9	69.6	70.3	78.2	77.0	124.9	107.4	105.4
3 weeks	81.0	70.4	79.9	80.6	79.8	113.1	113.7	97.4
5 weeks	86.3	75.8	80.4	82.8	79.5	128.4	133.1	117.5
7 weeks	77.4	71.9	76.8	74.3	69.2	117.5	104.0	90.0
9 weeks	84.7	73.9	75.6	72.1	68.1	122.6	114.0	108.5
Series IV								
2 weeks	...	...	79.8	80.9	78.6	137.2	139.5	125.3
3 weeks	...	...	81.3	80.5	80.6	121.7	136.0	124.8
5 weeks	...	...	80.3	78.0	80.2	134.3	129.3	101.1
7 weeks	...	...	79.5	77.1	77.6	137.5	121.0	117.3
9 weeks	...	...	93.0	86.9	84.6	128.1	124.0	109.1

striction of these normal young men to 0.23 mg. of thiamine per 1,000 Cal. had no significant effect on these typical measures of psychomotor speed, coordination and accuracy, whether measured in rest or in work.

Miscellaneous observations

In series I blood ketones were measured before and after work. The changes induced by the work were small and the results on the two different levels of thiamine intake disclosed no differences as large as the experimental error.

Blood hemoglobin, estimated every second week in all series, was remarkably constant on the average. In series I and II the average values were almost identical for the periods on low and high thiamine intake. In series III there was a tendency toward a slight rise in hemoconcentration as time went on (averages for first 3 weeks, 14.55; last 3 weeks, 15.00 gm. per 100 cc.).

Strength tests with grip, leg, and back dynamometers showed nothing but gradual and irregular training effects unrelated to the thiamine intake.

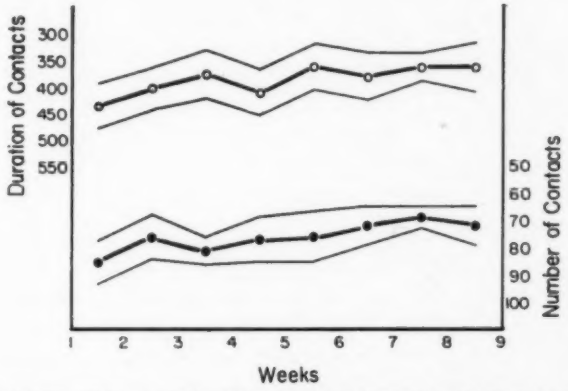

Fig. 1 Motor control in series IV during the first work period each week. The areas between the outer lines for duration of contacts and number of contacts enclose the standard deviations of the average scores plotted. High scores indicate poor performance.

Thiamine excretion

Thiamine excretion was not measured in series I. In series II the average 24-hour excretion of thiamine in the fourth and fifth weeks was 101 μg. on 1.59 mg. per day (0.53/1000 Cal.) and 291 μg. on 2.59 mg. per day (0.86/1000 Cal.).

In series III the 24-hour thiamine excretion averaged 106 μg. in the second week on 1.0 mg. per day intake (0.33 mg. per 1,000 Cal.) and remained at about that general level throughout; at the tenth week the average output was 92 μg. Individual

variations in single measurements varied from 38 to 151 μg. but the averages for the different individuals were fairly constant; for the last 8 weeks the individual average excretions were: 89, 107, 80 and 90 μg. per 24 hours. Saturation tests at the end with 4.11 mg. of thiamine chloride (orally) gave 7 to 10% recoveries except in a test on one man in which 26% of the test dose was excreted.

Figure 2 shows the average intakes and excretions of thiamine in series IV. Saturation tests on the subjects in series IV were made at the end of the experiment. The average recovery of the test dose was 6.4%.

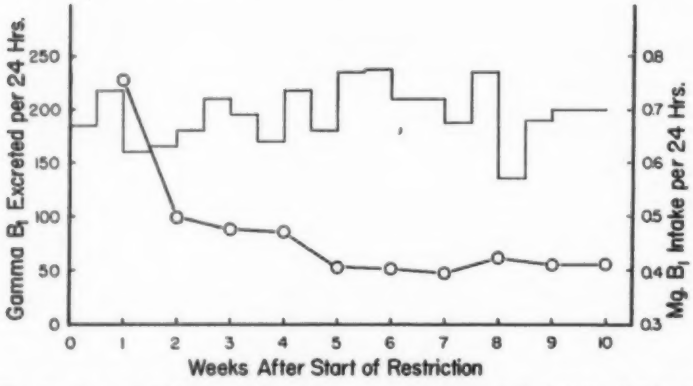

Fig. 2 Average 24-hour urinary excretion and average daily intake of thiamine in series IV. Excretion shown by circles.

DISCUSSION

The thiamine intakes listed in this paper are in terms of thiochrome analyses. These averaged 10.3% (standard deviation ± 6.7%) more than the values calculated from the most recent tables (Bowes and Church, '40; Booher et al., '42; Cheldelin and Williams, '42). Checks with meat samples by the rat growth method likewise indicated that the thiochrome method as used here slightly overestimates the true thiamine values. Accordingly we can say that the intakes were not more than stated and may have been 5 to 10% less.

The pyruvate values in the present experiments are of particular interest in view of the belief that an unusual rise in blood pyruvate in exercise (Lu and Platt, '39) or after glucose ingestion (Williams et al., '40, '41, '42, '43) is a specially sensiitve indicator of "hidden" or "latent" thiamine deficiency. It will be noted that the Mayo Clinic group found the maximum effect in their deficient subjects to be at 60 minutes after glucose. In our subjects at this time the blood pyruvate was uniformly lower than during rest on 0.23 mg. of thiamine per 1,000 Cal.

So far as comparisons can be made it appears that the per cent of dietary thiamine excreted in the urine was in reasonable agreement with expectations from other work (cf. Melnick, Field and Robinson, '39; Jolliffe et al., '39; Carden, Province and Ferrebee, '40; Williams et al., '42, '43). These comparisons raise a question: Should we consider the absolute amount of thiamine excretion or should we emphasize the proportion of dietary thiamine excreted? This may be important in comparing our subjects, who lived at a level of slightly over 3,000 Cal. per day, with those of Williams et al. ('43), who were maintained on a much lower level. In any case it appears that the excretions in series IV were in the range generally considered "deficient" (cf., e.g., Wang and Yudkin, '40; Elsom et al., '42; Melnick, '42; Melnick and Field, '42).

Of all the functions and characteristics measured in these studies the only evidence of any deficiency was in the urinary excretions. However. many authorities would consider the thiamine intake grossly deficient in series IV and at least moderately deficient in series III.

Estimation of the thiamine requirement demands definition of the bases on which the "requirement" is assessed. Most estimates for man have relied on one or more of the following criteria: (1) the appearance or removal of various signs or symptoms of disease; (2) the excretion of thiamine with or without a "saturation" test dose of the vitamin; (3) analogy with the results of animal studies, particularly growth rates.

The problem of the thiamine requirement of man is no longer chiefly concerned about the amount needed to prevent or to cure beri-beri. The "sub-clinical deficiency states" are, by definition, characterized by the absence of well-marked signs and symptoms. Vague subjective complaints and psychological alterations do not lend themselves to measurement and adequate control.

Thiamine excretion is undoubtedly related to the thiamine intake and "stores" in the body and this measurement is useful for many purposes. However, true adequacy or deficiency is not indicated by this means except by special definition. The difficulty is illustrated by the difference in interpretation of excretion levels by Wang and Yudkin ('40) and Williams et al. ('43), on the one hand, and Holt ('43) on the other.

There is now ample evidence that different animal species vary widely in thiamine requirements. Computations for man from experiments on the dog or rat may be grossly erroneous.

The conclusions to be drawn from any set of experiments are always somewhat limited to the conditions and materials of the experiment. This limitation is unusually narrow in vitamin studies at present because of the large number of factors which may play a role. Age, sex, climate, metabolic activity, disease and other foodstuffs may all contribute to the quantitative result. The duration of the period of subsistence is undoubtedly of importance.

The limitations in the present experiments are clearly recognized. We can conclude only that normal healthy young men living at around 3,000 Cal. per day on an ordinary balance of foodstuffs with an adequate supply of other vitamins are not benefitted, in any demonstrable way, by a thiamine intake of more than 0.23 mg. per 1,000 Cal. over a period of some 3 months. The possibility was not ruled out that less than 0.23 mg. would suffice or that deficiency might eventually appear on the 0.23 mg. regime.

In previous studies (Keys and Henschel, '42) we could find no physiological reason for increasing the thiamine intake of soldiers above the garrison ration level of about 0.43 mg. per

1,000 Cal. This finding was confirmed by Simonson and Enzer ('42) and was further confirmed on a broader basis in series I and II reported here.

SUMMARY AND CONCLUSIONS

1. Thiamine intake was limited and measured in four series of experiments. Each experiment lasted 10 to 12 weeks and involved four normal young men subjects. The diet was adequate except for thiamine and balance was maintained at 3050 ± 200 Cal. per day. Two control series were run in which all conditions and tests were applied identically but the subjects received in addition 1 mg. of thiamine daily.

2. Average thiamine intakes studied were, in milligram per 1,000 Cal., 0.63, 0.53, 0.33, and 0.23 as determined by thiochrome analyses on all food. Calculated values were 10.3%, $\sigma = \pm 6.7\%$, less than the thiochrome analyses.

3. Excretion of thiamine in the urine averaged, after a few weeks, about 10% of the dietary thiamine on an intake of 0.33 mg. per 1,000 Cal., and about 7% on 0.23 mg. Saturation tests gave similar results.

4. All subjects adhered to a fixed regime of weekly work performance. Blood and other examinations were made at regular intervals. All conditions and experiments were rigidly standardized. Tests covered simple strength, responses during brief exhausting work, prolonged severe work, and in recovery, psychomotor tests of speed and coordination, and glucose tolerance.

5. General and clinical observations and subjective reports were analyzed. Electrocardiographic and roentgenographic studies were made. Variables measured in rest, work, and recovery included heart rates, blood pyruvate, lactate, glucose and hemoglobin, and the psychomotor performances.

6. All results are in agreement that, for the periods studied, no benefit of any kind was observed to be produced by an intake of more than 0.23 mg. of thiamine per 1,000 Cal. At this level of intake muscular, neuromuscular, cardiovascular, psychomotor and metabolic functions were in no way limited by the

thiamine restriction. Clinical signs, subjective sensations and state of mind, and behavior were likewise apparently unaffected by thiamine intakes from 0.96 to 0.23 mg. per 1,000 Cal.

7. From the present work no conclusions are drawn as to requirements for men during more prolonged periods, or for women, children, the aged, or for states of disease or injury.

ACKNOWLEDGMENTS

We are indebted to many individuals for assistance in the onerous routine of this work. We are particularly glad to record our indebtedness to Miss Angie Mae Sturgeon, Head Technologist; Miss Ann Warenmaa and Miss Evelyn Pearson, Dietitians; Dr. Harold Loeb, Mr. Howard Condiff, and Mr. Frank Kurtz, Assistant Chemists; Miss Catherine Carroll, Technician; and Mr. Ersal Kindel, Laboratory Mechanic.

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STUDIES OF THE AVERAGE AMERICAN DIET

II. RIBOFLAVIN, NICOTINIC ACID AND PANTOTHENIC ACID CONTENT

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(Received for publication April 20, 1943)

The rapid accumulation of knowledge regarding the B vitamin contents of foods has made it possible to evaluate more properly than was previously possible the actual amount of these foods entering into the average American diet. These studies are especially timely at present in view of their importance to national nutrition.

Such an appraisal has already been made for thiamine (Lane, Johnson and Williams, '42). The present paper represents an extension of the above work, including the vitamins riboflavin, nicotinic acid and pantothenic acid.[2]

SELECTION OF FOODS

As in the thiamine studies (Lane, Johnson and Williams, '42), we have included assays of the more important foodstuffs in the American diet in proportions corresponding to the annual per capita consumption of each in the period 1934–1937.[3] Due to the increased scope of the present work

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[2] The work here presented is part of a study originally undertaken in the private laboratory of R. R. Williams, Summit, New Jersey, but which due to war interruptions was concluded at the University of Texas. The plan of the first paper of this series (Lane, Johnson and Williams, '42) has been followed in this one.

[3] This dietary does not reflect changes incident to the war period among which is the progressive enrichment of bread and flour.

the assays of mixed diets, as reported for thiamine, have been omitted; only assays of individual foods are reported here.

EXPERIMENTAL

The methods of sampling foods, preparing extracts and assaying for the various B vitamins have been described previously in detail (Cheldelin, Woods and Williams, '43; Cheldelin and Williams, '42).

Riboflavin, nicotinic acid and pantothenic acid content of individual foodstuffs

Tables 1–4 contain assays of all articles of foods studied, for each of the three vitamins listed above.

Wherever replicate samples have been assayed, either in this or previous papers (Cheldelin and Williams, '42; Cheldelin, Woods and Williams, '43) the values in tables 1–4 represent the average of the values obtained. The individual assays were obtained in many cases upon samples of different varieties and from different locations, in order to minimize differences in vitamin content due to these factors. Such environmental or varietal differences may sometimes be significant but are usually not great, as may be seen from an examination of the riboflavin assay values in table 5. Similar results have been obtained for thiamine and riboflavin in grains (Conner and Straub, '41; Nordgren and Andrews, '41).

Cooking waters and meat juices have been included as part of the cooked foods assayed except in a few cases where the cooking waters were assayed separately and discarded. The values in tables 1–4 therefore tend to represent maximum amounts of the various vitamins present after cooking.

Principal contributors of riboflavin, nicotinic acid and pantothenic acid to the average American diet

Riboflavin. As was pointed out for thiamine (Lane, Johnson and Williams, '42) the wide variations in caloric contributions of different foods to diets often cause the total vita-

min contributions of these foods to be quite different than would be expected on the basis of relative vitamin contents alone. Thus white bread, even though a relatively poor source of riboflavin, is second only to milk as a total contributor of riboflavin to the diet. Eggs are third in importance, and potatoes are a poor fourth. Liver is fifth in order despite its relatively high content of riboflavin. Dairy products as a class contribute nearly half of the total riboflavin supply to the diet, meats supply about one-fifth, and cereals, as well as fresh fruits and vegetables, each furnish about one-sixth of the total.

If the white bread in the diet were enriched to 0.7 mg. per pound (equal to 1.54 μg. per gram) as provided by Food Distribution Order No. 1 which is now in effect, the total supply of riboflavin in the diet would be 1.61 mg. per 2500 calories, representing slightly less than a 12% increase in the present intake. This is much less substantial than is the case for thiamine (Lane, Johnson and Williams, '42) or for nicotinic acid, as indicated below.

The total calculated daily intake of 1.4 mg. of riboflavin is in fair agreement with the estimate of approximately 1.7 mg. by Sherman ('42). These values, on the other hand, are appreciably lower than estimates of normal human requirement (Elvehjem, '41; Sebrell, Butler, Wooley and Isbell, '41; Williams, '42). If these estimates are correct, it seems necessary that we should either greatly increase the use of dairy products, in order to make the riboflavin supply adequate, or resort to a relatively heavy fortification of some staple with riboflavin.

Nicotinic acid. Flesh foods as a class contribute approximately three-fifths of the total daily intake of 11 mg. of nicotinic acid. This large amount is considerably out of proportion to the fraction of the total of other B vitamins supplied by flesh foods. The individual important contributors are beef, pork and fish, which together furnish about two-fifths of the total nicotinic acid consumed. White bread, which furnishes

TABLE 1

Yield of riboflavin per 100 gm. of foods as purchased.

FOOD	EDIBLE PORTION	MODE OF COOKING	CHANGE IN WEIGHT IN COOKING	RIBOFLAVIN					REMARKS
				Raw edible portion	Cooked edible portion	Total loss [3]	Yield of riboflavin in μg. Per 100 gm. raw edible portion [4]	Per 100 gm. as purchased [4]	
Cereal products	% [1]		% E. P. [2]	μg./gm.	μg./gm.	%			
White bread	100	None	..		0.90			90	Approx. 2% milk solids
Rye bread	100	None	..		0.72			72	So labeled; no milk
Whole wheat bread (100%)	100	None	..		1.1			110	So labeled; no milk
Whole wheat bread	100	None	..		0.67			67	So labeled; no milk
Corn meal	100	Mush	+370		0.13			63	
Oatmeal	100	Porridge	+300		0.27			110	
Rice, white	100	Steamed	+200		0.20			60	Cooking water absorbed
Rice Krispies	100	None	..		0.47		47	47	
Dairy products									
Milk	100	None	..	1.7				170	Raw, commercial sample
Milk	100	Heated in glass dish	0	1.7	0.87	48	87	87	Raw, commercial sample
Eggs	86	Scrambled	−11	4.4	3.2	35	290	260	60–65 gm./egg
Eggs	86	Poached	0	4.4	4.4	0	440	380	60–65 gm./egg
Cheese, American	100	None	..	5.9			590	590	
Meats									
Beef									
Round	96	Fried	−25	2.2	2.3	22	170	160	Juices included
Liver	100	Fried	−13	30	35	0	3000	3000	Juices included
Heart	100	Steamed	−13	8.9	10	0	890	890	Cooking water used
Pork									
Bacon	100	Fried	−50	3.1	5.6	9	280	280	Fat discarded
Ham	84	Fried	−42	2.6	3.6	19	210	180	Fat discarded
Loin chops	80	Fried	−37	2.3	2.7	26	170	140	Juices included
Mutton									
Shoulder	75	Steamed	0	2.5	2.5	0	250	190	Cooking water used
Veal									
Chop	80	Fried	−29	1.4	2.0	0	140	110	Juices included
Chop	80	Steamed	0	1.4	1.4	0	140	110	Cooking water used
Lamb									
Leg	80	Roasted	−20	2.5	3.1	0	250	200	Juices included
Chicken									
Leg	75	Fried	−27	2.6	3.5	3	250	190	Juices included
Breast	75	Fried	−7	1.3	1.4	0	130	100	Juices included
Fish									
Salmon	100	Fried	−13	1.4	1.7	0	140	140	Juices included
Salmon, canned	100	None	..		1.5		150	150	Can liquor used
Halibut	100	Steamed	0	0.44	0.44	0	44	44	Cooking water used
Halibut	100	Fried	−17	0.87	1.0	22	68	68	Juices used
Vegetables, cooked									
Asparagus, canned	100	None	..		1.0	0	100	100	Can liquor used
Beans, canned	100	None	..		0.38	0	38	38	Can liquor used
Beans, lima	100	Steamed	..	1.3	1.3	0	130	130	Cooking water used
Beans, snap, canned	90	None	..		0.52	0	52	47	Can liquor used
Beets	55	Boiled, covered kettle	−7	0.38	0.34	18 [5]	31	17	Tops removed. Cooked.
Beets	55	Steamed	0	0.61	0.61	0	61	61	Cooking water used
Beet greens	100	Steamed	0	2.2	1.9	14	190	190	Cooking water used
Cabbage	80	Steamed	0	0.57	0.48	16	48	38	Cooking water used
Cauliflower	60	Steamed	0	0.61	0.52	15	52	31	Cooking water used

Beans, snap, canned	90	None	..		0.52	0	52	47	Can liquor used
Beets	55	Boiled, covered kettle	−7	0.38	0.34	18 [6]	31	17	Tops removed. Cooked.
Beets	55	Steamed	0	0.61	0.61	0	61	61	Cooking water used
Beet greens	100	Steamed	0	2.2	1.9	14	190	190	Cooking water used
Cabbage	80	Steamed	0	0.57	0.48	16	48	38	Cooking water used
Cauliflower	60	Steamed	0	0.61	0.52	15	52	31	Cooking water used
Corn, canned	100	None	..		0.80		80	80	Can liquor used
Okra	88	Steamed	0	1.0	1.0	0	100	88	Cooking water used
Onions	94	Fried	−77	0.24	1.0	26	18	17	
Peas, canned	100	None	0		1.0	0	100	100	Can liquor used
Peas, dried	100	Boiled	+200	1.8	0.60	0	180	180	Cooking water absorbed
Potatoes	85	Boiled, covered dish	+15	0.50	0.25	43 [6]	28	24	Cooking water discarded
						0	50	43	Cooking water used
Potatoes	85	Steamed	+5	0.50	0.42	12 [6]	44	37	Cooking water discarded
						7	47	40	Cooking water used
Pumpkin, canned	100	None	..		0.62		62	62	
Sauerkraut	100	Steamed	0	0.40	0.38	5	38	38	Cooking water used
Spinach	83	Steamed	0	2.2	1.6	27	160	130	Cooking water used
Sweet potatoes	100	Baked	−23	0.41	0.46	13	36	36	Skins used
Tomatoes, canned	100	None	..		0.32	0	32	32	Can liquor used
Turnips	66	Boiled, covered dish	+3	0.42	0.38	10 [6]	38	25	Cooking water discarded
						0	42	28	Cooking water used
Vegetables, raw									
Cabbage	80			0.57			57	46	
Carrots	63			0.50			50	32	
Lettuce	95			0.27			27	26	
Tomatoes	97			0.37			37	36	
Fruits, cooked									
Apples	80	Boiled, open dish	−33	0.17	0.19	25	13	10	Made into applesauce, including liquor
Pears, canned	100	None	..		0.13		13	13	Can liquor used
Prunes, canned	97	None	..		0.25		25	24	Can liquor used
Prunes, dried	83	None	..				54	45	
Fruits, raw									
Apples	91			0.17			17	15	
Bananas	68			0.56			56	38	
Canteloup	50			0.26			26	13	
Grapefruit	66			0.30			30	20	
Oranges	63			0.47			47	30	
Peaches, frozen	100			0.17			17	17	
Raisins	100			0.29			29	29	
Strawberries	97			0.34			34	33	
Watermelon	59			0.69			69	34	
Miscellaneous									
Peanuts	69			1.1			110	76	
Chocolate	100			2.4			240	240	
Molasses	100			0.62			62	62	

[1] Per cent of "as purchased weight" after discarding inedible portions such as bones, tops, peelings, etc.
[2] Calculated to represent the change in weight of edible portion only. The values are only approximate when the inedible portion is discarded after cooking, as with bones in roasts, cobs from sweet corn, or skins from potatoes baked whole.
[3] Total loss does not include vitamin loss in cooking liquors unless specified under footnote 5.
[4] Includes vitamin in cooking waters unless otherwise indicated in "remarks" column.
[5] Total loss includes thermal loss and loss in cooking liquors.

TABLE 2

Yield of nicotinic acid per 100 gm. of foods as purchased.

FOOD	EDIBLE PORTION	MODE OF COOKING	CHANGE IN WEIGHT IN COOKING	NICOTINIC ACID					REMARKS
				Raw edible portion	Cooked edible portion	Total loss [2]	Yield of nicotinic acid in micrograms		
							Per 100 gm. raw edible porion [4]	Per 100 gm. as purchased [4]	
	% [1]		% E. P. [3]	γ/gm.	γ/gm.	%			
Cereal products									
White bread	100	None	..		6.0 (7.0) [6]			600 (700) [6]	Approx. 2% milk solids
Rye bread	100	None	..		9.2 (11)			920 (1100)	So labeled; no milk
Whole wheat bread (100%)	100	None	..		28 (40)			2800 (4000)	So labeled; no milk
Whole wheat bread	100	None	..		9.1 (11)			910 (1100)	So labeled; no milk
Corn meal	100	Mush	+370		1.9 (2.5)	0	900	900 (1200)	
Oatmeal	100	Porridge	+300		2.0 (2.2)	0	800	800 (880)	
Rice	100	Steamed	+200	5.9	2.0	0	590	590	Cooking water absorbed
Rice Krispies	100	None	..		6.9			690	
Dairy products									
Milk	100	None	..	0.66			66	66	Raw, commercial sample
Eggs	86	Scrambled	−11	0.67	0.57	25	51	44	60–65 gm./egg.
Cheese, American	100	None	..	0.2			20	20	
Meats									
Beef									
Round	96	Fried	−25	45	56	6	4200	4100	Juices included
Liver	100	Fried	−13	120	140	0	12000	12000	Juices included
Heart	100	Steamed	−13	83	82	14	7100	7100	Cooking water used
Pork									
Bacon	100	Fried	−50	42	84	0	4200	4200	Fat discarded
Ham	84	Fried	−42	33	57	0	3300	2800	Fat discarded
Loin chops	80	Fried	−37	60	51	46	3200	2600	Juices included
Mutton									
Shoulder	75	Steamed	0	40	40	0	4000	3000	Cooking water used
Lamb leg	80	Roasted	−20	75	76	19	6100	4900	Juices included
Veal									
Chop	80	Fried	−29	71	87	13	6200	4900	Juices included
Chop	80	Steamed	0	71	65	9	6500	5200	Cooking water used
Chicken									
Leg	75	Fried	−27	38	44	15	3200	2400	Juices included
Breast	75	Fried	−7	94	100	0	9400	7100	Juices included
Fish									
Salmon	100	Fried	−13	64	69	7	6000	6000	Juices included
Salmon, canned	100	None	..		64		6400	6400	Can liquor used
Halibut	100	Steamed	0	110	104	6	11000	10000	Cooking water used
Halibut	100	Fried	−17	110	130	2	11000	11000	Juices included
Vegetables, cooked									
Asparagus, canned	100	None	..		7.4		740	740	Can liquor used
Beans, snap, canned	90	None	..		2.4		240	220	Can liquor used
Beets	55	Boiled	−7	5.6	2.8	54 [5]	260	140	Tops removed. Cooked. H_2O discarded

Vegetables, cooked									
Asparagus, canned	100	None			7.4		740	740	Can liquor used
Beans, snap, canned	90	None			2.4		240	220	Can liquor used
Beets	55	Boiled	−7	5.6	2.8	54 [5]	260	140	Tops removed. Cooked. H_2O discarded
						18	460	250	Cooked, H_2O used
Beets	55	Steamed	0	6.5	2.5	61	250	140	Cooking water used
Beet greens	100	Steamed	0	6.0	5.8	4	580	580	Cooking water used
Cabbage	80	Steamed	0	2.1	1.7	19	170	140	Cooking water used
Carrots	63	Steamed	0	3.1	3.1	0	310	200	Cooking water used
Cauliflower	60	Steamed	0	5.7	5.2	9	520	310	Cooking water used
Corn, canned	100	None	0		13		1300	1300	Can liquor used
Okra	88	Steamed	0	7.1	7.1	0	710	630	Cooking water used
Peas, canned	100	None	0		7.8		780	780	Can liquor used
Peas, dried	100	Boiled	+200	28 (35) [6]	9.3	0	2800 (3500) [6]	2800 (3500) [6]	Cooking water absorbed
Potatoes	85	Boiled	+15	14	9.3	27 [5]	1000	850	Cooking water discarded
						0	1400	1200	Cooking water used
Potatoes	85	Steamed	+5	8.4	5.5	31 [5]	580	490	Cooking water discarded
						0	840	710	Cooking water used
Pumpkin, canned	100	None	0		5.4		540	540	
Sauerkraut	100	Steamed	0	1.7	1.6	6	160	160	Cooking water used
Spinach	83	Steamed	0	5.1	4.3	16	430	360	Cooking water used
Tomatoes, canned	100	None	0		4.7		470	470	Can liquor used
Turnips	66	Boiled	+3	9.7	5.6	40 [5]	580	380	Cooking water discarded
						0	970	640	Cooking water used
Vegetables, raw									
Cabbage	80			2.1			210	170	
Carrots	63			3.1			310	200	
Lettuce	95			2.5			250	240	
Tomatoes	97			4.7			470	460	
Fruits, cooked									
Pears, canned	100	None	0		1.7		170	170	Can liquor used
Prunes, canned	97	None	0		2.4		240	230	Can liquor used
Fruits, raw									
Apples	91			0.81			81	74	
Bananas	68			5.8			580	400	
Canteloup	50			10			1000	500	
Grapefruit	66			2.1			210	140	
Oranges	63			3.0			300	190	
Peaches, frozen	100			3.3			330	330	
Raisins	100			2.9			290	290	
Strawberries	97			2.2			220	210	
Watermelon	59			2.4			240	140	
Miscellaneous									
Peanuts	69			86			8600	5900	
Chocolate	100			11			1100	1100	
Molasses	100			39			3900	3900	

[1] See footnotes 1, 2, 3, 4 and 5 to table 1.

[6] Nicotinic acid values in parentheses represent assays of acid hydrolyzed extracts.

TABLE 3

Yield of pantothenic acid per 100 gm. of foods as purchased.

FOOD	EDIBLE PORTION	MODE OF COOKING	CHANGE IN WEIGHT IN COOKING	PANTOTHENIC ACID					REMARKS
				Raw edible portion	Cooked edible portion	Total loss [3]	Yield of pantothenic acid in micrograms		
							Per 100 gm. raw edible portion [4]	Per 100 gm. as purchased [4]	
	% [1]		% E. P. [2]	μg./gm.	μg./gm.	%			
Cereal products									
White bread	100	None	..		4.6			460	Approx. 2% milk solids
Rye bread	100	None	..		5.1			510	So labeled; no milk
Whole wheat bread (100%)	100	None	..		5.7			570	So labeled; no milk
Whole wheat bread	100	None	..		4.2			420	So labeled; no milk
Corn meal	100	Mush	+370		0.66	0	310	310	
Oatmeal	100	Porridge	+300		3.2	0	1300	1300	Cooking water absorbed
Rice	100	Steamed	+200	3.5	1.2	0	350	350	Cooking water absorbed
Rice Krispies	100	None			3.4			340	
Dairy products									
Milk	100	None		2.9			290		Raw, commercial sample
Eggs	86	Poached	0	14	14	0	1400	1200	60–65 gm./egg
Cheese, American	100	None	..	2.3			230	230	
Meats									
Beef									
Round	96	Fried	−25	4.9	4.4	32	330	250	Juices included
Liver	100	Fried	−13	76	75	14	6500	6500	Juices included
Heart	100	Steamed	−13	20	14	37	1300	1300	Cooking water used
Pork									
Bacon	100	Fried	−50	5.2	9.2	12	460	460	Fat discarded
Ham	84	Fried	−42	5.0	8.6	0	500	320	Fat discarded
Loin chops	80	Fried	−37	4.4	4.8	31	300	240	Juices included
Mutton									
Shoulder	75	Steamed	0	4.3	4.3	0	430	320	Cooking water used
Lamb leg	80	Roasted	−20	6.0	6.8	12	530	420	Juices included
Veal									
Chop	80	Fried	−29	1.9	2.3	14	160	130	Juices included
Chicken									
Leg	75	Fried	−27	6.2	6.1	28	450	340	Juices included
Breast	75	Fried	−7	5.3	4.9	14	460	350	Juices included
Fish									
Salmon	100	Fried	−13	6.6	7.6	0	660	660	Juices included
Salmon, canned	100	None	..		4.5		450	450	Can liquor used
Halibut	100	Steamed	0	1.5	1.5	0	150	150	Cooking water used
Halibut	100	Fried	−17	1.5	1.8	0	150	150	Juices included
Vegetables, cooked									
Asparagus, canned	100	None	0		1.4		140	140	Can liquor used
Beans, lima	100	Steamed	0	8.3	6.1	26	610	610	Cooking water used
Beans, snap, canned	90	None	0		1.1		110	99	Can liquor used
Beets	55	Boiled	−7	1.3	1.1	24 [5]	99	54	Tops removed. Cooked. H$_2$O discarded

Vegetables, cooked								
Asparagus, canned	100	None	0		1.4		140	140	Can liquor used
Beans, lima	100	Steamed	0	8.3	6.1	26	610	610	Cooking water used
Beans, snap, canned	90	None	0		1.1		110	99	Can liquor used
Beets	55	Boiled	−7	1.3	1.1	24 [5]	99	54	Tops removed. Cooked. H_2O discarded
						18	107	59	Cooked. H_2O used
Beets	55	Steamed	0	1.1	0.68	38	68	38	Cooking water used
Beet greens	100	Steamed	0	1.4	0.96	36	96	96	Cooking water used
Cabbage	80	Steamed	0	1.8	1.3	28	130	110	Cooking water used
Carrots	63	Steamed	0	2.5	2.3	10	230	140	Cooking water used
Cauliflower	60	Steamed	0	9.2	8.9	3	890	540	Cooking water used
Corn, canned	100	None	0		3.2		320	320	Can liquor used
Okra	88	Steamed	0	2.1	2.1	0	210	180	Cooking water used
Peas, canned	100	None	0		1.3		130	130	Can liquor used
Peas, dried	100	Boiled	+200		4.3		1300	1300	Cooking water absorbed
Potatoes	85	Boiled	+5	3.2	1.9	40 [5]	190	160	Cooking water discarded
						0	320	270	Cooking water used
Pumpkin, canned	100	None	0		1.3		130	130	
Sauerkraut	100	Steamed	0	0.57	0.57	0	57	57	Cooking water used
Spinach	83	Steamed	0	1.8	1.7	6	170	140	Cooking water used
Tomatoes, canned	100	None	0		4.1		410	410	Can liquor used
Turnips	66	Boiled	+3	1.4	0.8	41 [5]	83	55	Cooking water discarded
						8	130	86	Cooking water used
Vegetables, raw									
Cabbage	80			1.8			180	140	
Carrots	63			2.5			250	160	
Lettuce	95			1.1			110	100	
Tomatoes	97			3.7			370	360	
Fruits, cooked									
Pears, canned	100	None			0.20		20	20	Can liquor used
Prunes, canned	97	None			0.43		43	42	Can liquor used
Fruits, raw									
Apples	91			0.60			60	55	
Bananas	68			1.8			180	120	
Canteloup	50			2.3			230	115	
Grapefruit	66			2.9			290	190	
Oranges	63			3.4			340	210	
Peaches, frozen	100			1.7			170	170	
Raisins	100			0.90			90	90	
Strawberries	97			2.6			260	250	
Watermelon	59			3.1			310	180	
Miscellaneous									
Peanuts	69			25			2500	1700	
Chocolate	100			1.9			190	190	
Molasses	100			2.6			260	260	

See footnotes 1, 2, 3, 4 and 5 to table 1.

TABLE 4

Riboflavin, nicotinic acid and pantothenic acid contributions from various foods to make a total of 2500 calories.

FOODS	GM.	CAL.	CAL. % OF TOTAL	RIBOFLAVIN		NICOTINIC ACID		PANTOTHENIC ACID	
				µg.	% of total	*µg.*	% of total	*µg.*	% of total
White bread (includes some sugar and fat)	260 [1]	679	27.3	240	16.0	1600 (1800) [2]	13.9 (15.4) [2]	1200	26.2
Rye bread	4	9	0.4	3	0.2	37 (44) [2]	0.3 (0.4) [2]	20	0.4
Whole wheat bread	6	16	0.6	4	0.3	55	0.5	25	0.5
Corn meal	11	41	1.6	7	0.5	99 (120) [2]	0.9 (1.0) [2]	34	0.7
Oatmeal	4	17	0.7	4	0.3	32 (35) [2]	0.3	52	1.1
Prepared breakfast cereals	6	20	0.8	3	0.2	41	0.4	17	0.4
Rice	5	16	0.6	3	0.2	30	0.3	18	0.4
Sugar (apart from bread)	97	384	15.3	0	0	0	0	0	0
Total cereal products	393	1182	47.3	260	18	1900 (2100) [2]	17 (18) [2]	1360	30
Milk	320	222	8.9	540	37.3	210	1.8	930	20.3
Eggs	34	48	1.9	120	8.3	15	0.1	410	9.0
Cheese	8	30	1.2	47	3.2	1.6	0	18	0.4
Butter	20	148	5.9	2	0.1	0	0	0	0
Total dairy products	107	448	17.9	710	49	230	1.9	1360	30
Bacon (including fat pork)	7	43	1.7	19	1.3	300	2.7	32	0.7
Pork (other than bacon)	39	155	6.2	62	4.3	1100	9.8	130	2.8
Mutton and lamb	6	16	0.6	13	0.8	240	2.2	22	0.5
Beef	46	104	4.2	74	5.1	1900	16.9	140	3.1
Veal	7	10	0.4	9	0.6	360	3.2	11	0.2
Poultry	15	21	0.8	21	1.5	750	6.7	52	1.1
Fish	20	14	0.6	20	1.4	1600	14.2	70	1.5
Liver and other organs	3	3	0.1	60	4.1	300	2.7	150	3.3
Total flesh	143	366	14.6	280	19	6600	59	610	13
Fats and oils [3]	19	171	6.8	0	0	0	0	0	0
Asparagus, canned [4]	4	1	..	4	0.3	30	0.3	6	0.1
Beans, baked, canned [4]	7	7	0.3	3	0.2	25	0.2	13	0.3
Beans, lima, canned [4]	2	2	..	3	0.2	20	0.2	130	2.8
Beans, snap	11	4	0.1	5	0.3	24	0.2	11	0.2
Beets	4	1	..	1	0.1	10	0.1	2	0
Cabbage, boiled (inc. cauliflower)	15	3	0.1	5	0.3	35	0.3	50	1.1
Cabbage, raw [5]	5	1	..	2	0.1	10	0.1	7	0.1
Carrots, boiled	10	3	0.1	4 (cut.)	0.3	20	0.2	14	0.3
Carrots, raw [5]	3	1	..	1	0.1	9.3	0.1	5	0.1
Corn, canned [4]	13	13	0.5	10	0.7	170	1.5	42	0.9
Okra (including eggplant)	4	2	..	3	0.2	25	0.2	7	0.1
Onions	21	8	0.1	4	0.3	15	0.2	15	0.3

Cabbage, boiled (inc. cauliflower)	15	3	0.1	3		0.3	35		0.3	30		1.1
Cabbage, raw [6]	5	1	..	2		0.1	10		0.1	7		0.1
Carrots, boiled	10	3	0.1	4	(est.)	0.3	20		0.2	14		0.3
Carrots, raw [6]	3	1	..	1		0.1	9.3		0.1	5		0.1
Corn, canned [4]	13	13	0.5	10		0.7	170		1.5	42		0.9
Okra (including eggplant)	4	2	..	3		0.2	25		0.2	7		0.1
Onions	21	9	0.4	4		0.3	15	(est.)	0.1	25	(est.)	0.5
Lettuce	12	1	..	3		0.2	30		0.3	12		0.3
Parsnips and radishes	2	1	..	1		0.1						
Peas, canned [4]	13	7	0.3	13		0.9	100		0.9	17		0.4
Peas and beans, dried	7	24	1.0	11		0.8	140	(170) [2]	1.2	(1.5) [2]	65	1.4
Potatoes	138	100	4.0	41		2.9	950		8.4		220	4.8
Pumpkin, canned [4]	7	3	0.1	4		0.3	38		0.3		9	0.2
Sauerkraut	3	1	..	1		0.1	4.8		0		2	0
Spinach	6	1	..	8		0.6	22		0.2		8	0.2
Sweet potato	20	22	0.9	7		0.5	100	(est.)	0.9		180	(est.) 3.9
Tomatoes, canned [4]	18	4	0.2	5		0.3	85		0.7		74	1.6
Tomatoes, raw [5]	18	4	0.2	5		0.3	85		0.7		67	1.5
Turnips	4	1	..	1	(est.)	0.1	15		0.1		2	0
Total vegetables	347	219	8.8	145		10	2000		17		970	21
Apples, cooked	19	11	0.5	2		0.1	15	(est.)	0.1		12	(est.) 0.3
Apples, (raw, including pears) [6]	37	21	0.8	6		0.4	37		0.3		20	0.4
Bananas	25	16	0.6	10		0.7	100		0.9		30	0.7
Strawberries (including other berries)	5	2	..	2		0.1	11		0.1		13	0.3
Canteloup	7	1	..	1		0.1	35		0.3		8	0.2
Grapes	5	3	0.1									
Oranges and other citrus fruits	51	18	0.7	13		0.9	90		0.8		100	2.2
Peaches	10	5	0.2	2		0.1	33		0.3		17	0.2
Prunes, cooked	5	11	0.4	2		0.1	12		0.1		2	0
Raisins	2	6	0.2	0		0	6		0.1		2	0
Watermelon	15	2	0.1	5		0.3	21		0.2		27	0.6
Total fruits	181	96	3.9	43		3.0	360		3.2		230	4.9
Peanuts (including other nuts)	2	12	0.5	2		0.1	120		1.1		34	0.7
Chocolate	1	8	0.3	2		0.1	11		0.1		2	0
Total miscellaneous	3	20	0.8	4		0.2	130		1.2		36	0.7
Grand total	1193	2502	100.2	1440		99	11200	(11400) [2]	99		4600	99

[1] Sufficient white bread was included in the diet to account for the total average white flour consumption. Part of this, of course, appears as other bakery goods, macaroni, etc., in customary human diets.

[2] Nicotinic acid values in parentheses represent assays of acid hydrolyzed extracts.

[3] This does not include fats contained in meats. A portion of the total shortening appears in the bread, that amount being deducted from the average consumption of fats and oils.

[4] For convenience we used and analyzed canned product but it should not be inferred that the product is not used in customary diets in other ways.

[5] All foods other than fruits were cooked except when otherwise indicated.

TABLE 5

The riboflavin content of foods of different variety and source.

MATERIAL	SOURCE	BRAND OR VARIETY	NUMBER OR QUANTITY OF SPECIMENS TESTED	DRY WEIGHT	RIBOFLAVIN CONTENT EDIBLE PORTION	
					Fresh	Dry
				%	*μg./gm.*	*μg./gm.*
Apples	Washington	Delicious	9	15.0	0.15	1.00
	Washington	Delicious	6	14.0	0.13	0.93
	Washington	Rome Beauty	9	14.0	0.16	1.14
	New York	Greening	10	14.4	0.21	1.46
	New York	MacIntosh	10	13.8	0.17	1.23
	New Jersey	Baldwin	8	14.5	0.20	1.37
Asparagus, canned	California	Del Monte	16 oz.	6.4	0.62	9.7
	California	Del Monte	14 oz.	6.4	0.54	8.4
	?	Rob-Ford	18 oz.	6.0	1.07	18
Carrots	Texas	?	7	13.6	0.43	3.2
	California	?	15	13.5	0.50	3.7
Cheese	Wisconsin	Cheddar	3	74.0	6.2	8.4
	Wisconsin	Mild cream	1	65.0	5.4	8.3
	Wisconsin	Proc. cheddar	1	62.0	5.1	8.2
Eggs	Oregon	White Leghorn	12	25.8	2.2	8.5
	Oregon	White Leghorn	12	26.0	4.2	16
	Oregon	R. I. Red	12	26.0	4.5	18
	Oregon	R. I. Red	12	26.0	5.1	20
	New Jersey	White Leghorn	12	26.8	6.4	24
	New Jersey	White Leghorn	12	26.5	3.6	14
	New Jersey	R. I. Red	12	26.0	5.2	20
	Texas	White Leghorn	12	25.5	4.2	17
Lettuce	Arizona	Head	1	5.2	0.33	6.3
	California	Head	1	5.0	0.20	4.0
Onions	Texas	White	10	10.6	0.23	2.2
	Texas	Brown	4	12.5	0.24	1.9
Oranges	Florida	?	6	13.0	0.51	3.9
	California	Navel	6	16.0	0.42	2.6
Parsnips	Pennsylvania	?	6	24.4	0.63	2.6
	Texas	?	9	22.8	0.91	4.0
Peanuts	Brazil	?	70 gm.	98.4	0.98	1.00
	Virginia	?	70 gm.	98.0	1.12	1.14
Potatoes	Maine	Green Mtn.	6	16.6	0.36	2.2
	Idaho	Netted Gem	6	20.0	0.23	1.2
	Florida	Early Rose (new)	9	11.4	0.29	2.5
Raisins	California	Seedless	100 gm.	76	0.29	0.38
	California	Seeded	100 gm.	75	0.20	0.27
Sweet potatoes	S. Carolina	Yams	7	29.2	0.40	1.4
	New Jersey	White	7	36.2	0.45	1.2
Tomatoes	California	?	4	5.2	0.24	4.6
	California	?	4	6.8	0.24	3.5

about one-seventh, and potatoes with one-twelfth of the total, are the only other important contributors.

The present value of 11 mg. daily average intake of nicotinic acid rates as marginal or low when compared with the estimated requirements of this vitamin (Elvehjem, '41; Williams, '42). Enrichment of bread, on the other hand, has provided substantial increases in the average intake. The prevailing addendum of 10 mg. of nicotinic acid per pound of bread results in a total intake of approximately 17 mg. per 2500 calories.

Pantothenic acid. This vitamin, like thiamine, is more evenly distributed than riboflavin or nicotinic acid among the various classes of foods comprising the average American diet. Cereals, dairy products and fresh vegetables each furnish about one-fourth of the total supply of pantothenic acid. The individual important sources are white bread, milk, eggs and potatoes, in the order named.

Data regarding the nutritional significance and requirements of pantothenic acid are meager. The estimated daily requirements of 9 to 11 mg. by Gordon ('42) and of 11 mg. by Williams ('42) are far in excess of the actual average intake. Deficiencies of this vitamin, if they exist, do not appear to be due in large measure to milling losses during flour manufacture. Restoration by enrichment of bread with pantothenic acid would therefore result in only a slight increase in the daily supply.

CONCLUSIONS

The riboflavin, nicotinic acid and pantothenic acid contents of the average American diet, such as was consumed by the middle two-thirds or three-fourths of the population prior to the use of enriched bread and flour, are approximately 1.4 mg., 11 mg., and 4.9 mg., respectively, per 2500 calories. These values are apppreciably lower than the most generally supposed daily requirements.

Enrichment of bread and flour to meet prevailing standards increases the level of riboflavin, 12% to 1.6 mg.; that of nicotinic acid, 53% to 17 mg.

The principal contributors of these vitamins to ordinary diets are as follows in the order of their importance: for riboflavin, milk, white bread, eggs, potatoes and liver; for nicotinic acid, beef, lean pork, fish, white bread and potatoes; for pantothenic acid, white bread, milk, eggs and potatoes.

Tables are presented to permit the calculation of vitamin contents of other diets.

ACKNOWLEDGMENT

We are indebted to Dr. Roger J. Williams for his cooperation and many suggestions during this work. We wish to acknowledge, with thanks, the technical aid of Misses Margaret J. Hofer, Jean Taylor and Alethea M. Woods, as well as the financial aid of Research Corporation.

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THE MINERAL COMPOSITION OF THE ALBINO RAT AS AFFECTED BY CHLORIDE DEFICIENCY [1]

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(Received for publication April 14, 1943)

In a previous report (Voris and Thacker, '42) it was shown that the substitution of bicarbonate for chloride in the diets of rats served to diminish their growth, appetite, and gain of nitrogen and energy, and to increase their water consumption and heat production. Thus, the nutritional effects of a deficiency of dietary chloride are, in many respects, similar to those of deficiencies of sodium and potassium (Orent-Keiles, Robinson and McCollum, '37; Kahlenberg, Black and Forbes, '37; Orent-Keiles and McCollum, '41).

Recently, Greenberg and Cuthbertson ('42) found that rats fed a low chloride diet gained less in weight, and had lower blood chloride and higher total carbon dioxide contents than did rats which received a diet of normal chloride content. Since no significant difference was found between the pH values of the whole blood of the chloride deficient and the control animals, the alkalosis arising from the increased carbon dioxide content of the blood was, then, virtually compensated.

However, though the chloride ion is one of the chief constituents of the body fluids, little is known of the specific rôle of chloride in its relations to other mineral elements. In the present investigation, the metabolism of chloride, sodium, potassium, calcium, magnesium, and phosphorus in rats as affected by feeding a diet deficient in chloride has been studied.

[1] Authorized for publication on April 8, 1943, as paper no. 1176 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

EXPERIMENTAL

This study was carried out in conjunction with an experiment previously reported from this laboratory (Voris and Thacker, '42). The composition of the diets and details of experimental procedure were the same in both investigations and will not be repeated here.

The bodies, feces and urines of all the rats were analyzed for chloride, sodium, potassium, calcium, and magnesium, except that only chloride was determined on the urine of the chloride deficient rats on account of the loss of samples during the ashing process. Composite samples of the male and female bodies were analyzed for phosphorus.

The analytical methods used were as follows: chloride, Sendroy's titrimetric iodate method (Sendroy, '37); sodium, Barber and Kolthoff ('28, '29); potassium, the chloroplatinate separation with the potassium estimated by reducing the potassium chloroplatinate with powdered metallic magnesium, then determining the released chloride ion by Sendroy's titrimetric iodate method; calcium and magnesium, as outlined by Hillebrand and Lundell ('29); phosphorus, on the recombined rat bodies (ether extract recombined with the body residues) after wet digestion, by the volumetric method of the Association of Official Agricultural Chemists ('30).

Sodium, potassium, calcium, and magnesium were determined on aliquots of the diets, body residues, feces, and urines ashed overnight at a dull red heat (550° C.). Chloride was determined on the fresh urines, and on 2.5 gm. samples of the diets, body residues, and feces containing 5 ml. of a 20% solution of sodium carbonate, and ashed overnight at a dull red heat (550° C.).

Twelve rats (six male, six female), corresponding in age, weight and breeding to the paired rats used as experimental subjects, were killed and analyzed to represent the mineral composition of the experimental rats at the start of the feeding experiment.

The gain in each mineral during the experimental period, therefore, was determined by the difference between the com-

position of the rats at the end of the experiment and of the controls selected to represent these animals at the beginning of the experimental feeding. The average analyses for males and females were used separately.

RESULTS AND DISCUSSION

Table 1 summarizes the composition of the rations with respect to chloride, sodium, potassium, calcium, magnesium, nitrogen, energy and dry matter. Although the diets were not analyzed for phosphorus, the approximate phosphorus content was 0.153%. It was intended to have both the deficient and the control rations of similar mineral composition, except for chloride content. This objective was accomplished reasonably well except with reference to sodium.

Inasmuch as the growth data have already been adequately discussed by Voris and Thacker ('42), the data in table 2 are presented primarily to indicate the derivation of the calculated values in the following tables.

The average percentage composition of the seven male and five female rats of the normal and of the chloride deficient groups as to chloride, sodium, potassium, calcium, magnesium, and phosphorus is given in table 3. These values are also expressed as percentages of the fat-free tissue, and as millimoles per kilogram of body water. In addition, the retention of these minerals, computed by comparison of the composition of the bodies of the chloride deficient rats at the end with that representing their composition at the beginning of the experimental feeding, is recorded as total milligrams, as per cent of fat-free tissue gained, and as millimoles per kilogram of water gained.

Inspection of the standard errors reveals that when the mineral composition is expressed as a function of body weight or water content, the individual variation is surprisingly small, though the final live weight of the rats varied as much as 62 gm. Mineral composition or mineral retention has little significance unless correlated with body weight or with water content.

TABLE 1

Composition of rations.

RATION	NITROGEN	ENERGY	DRY MATTER	CHLORIDE	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM
	%	*Cals./gm.*	%	%	%	%	%	%
Normal chloride [1]	2.89	4.66	98.6	0.28	0.102	0.315	0.617	0.0381
Chloride deficient	2.89	4.66	98.6	0.02	0.120	0.305	0.624	0.0366

[1] Average of two batches.

TABLE 2

Growth data of normal control and chloride deficient rats for a 70-day period. Averages for seven males and five females.

	LIVE WEIGHT [1]	FAT-FREE TISSUE [1]	GAIN OF BODY TISSUE	GAIN OF FAT-FREE TISSUE	BODY WATER	GAIN OF WATER
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
♂ Control	176.7	158.7	113.0	100.7	113.8	70.3
♂ Chloride deficient	147.6	134.9	83.7	76.6	95.8	52.1
♀ Control	158.5	137.8	104.5	89.1	98.8	61.5
♀ Chloride deficient	129.1	114.4	75.8	66.4	81.1	44.7

[1] Does not include the contents of alimentary tract.

TABLE 3

Mineral constituents of the bodies of normal control and of chloride deficient rats. Averages for seven males and five females.[1]

	PER CENT OF LIVE WEIGHT [2]	PER CENT OF FAT FREE TISSUE	MILLIMOLES PER KILOGRAM BODY WATER	RETENTION DURING 70 DAYS		
				Total	Per cent of fat-free tisue gained	Millimoles per kilogram water gained
				mg.		
Chloride						
♂ Control	0.098 ± .002	0.109 ± .002	43.0 ± .65	102.2	0.101 ± .003	41.0 ± 1.05
♂ Chloride deficient	0.087 ± .002	0.096 ± .002	37.9 ± .62	57.5	0.075 ± .003	31.2 ± 1.15
♀ Control	0.092 ± .002	0.106 ± .003	41.7 ± .96	84.4	0.095 ± .003	38.8 ± 1.53
♀ Chloride deficient	0.085 ± .001	0.096 ± .001	38.3 ± .53	50.1	0.075 ± .002	31.5 ± .78
Sodium						
♂ Control	0.107 ± .002	0.119 ± .002	72.2 ± 1.13	118.2	0.117 ± .003	73.1 ± 1.85
♂ Chloride deficient	0.105 ± .0005	0.115 ± .0006	70.5 ± .40	84.5	0.110 ± .001	70.5 ± .77
♀ Control	0.107 ± .0008	0.124 ± .004	75.0 ± 2.27	111.3	0.126 ± .006	79.8 ± 3.96
♀ Chloride deficient	0.103 ± .002	0.116 ± .001	71.0 ± .56	74.7	0.113 ± .002	72.6 ± 1.20
Potassium						
♂ Control	0.268 ± .002	0.298 ± .003	106.4 ± 1.03	303.9	0.301 ± .005	110.4 ± 1.50
♂ Chloride deficient	0.261 ± .002	0.286 ± .002	103.0 ± .78	215.5	0.280 ± .004	105.6 ± 1.47
♀ Control	0.264 ± .004	0.303 ± .004	108.6 ± 1.54	273.5	0.308 ± .007	118.4 ± 6.38
♀ Chloride deficient	0.261 ± .002	0.294 ± .002	106.0 ± .50	194.3	0.293 ± .003	111.1 ± 1.07
Calcium						
♂ Control	1.20 ± .03	1.34 ± .03		1535	1.53 ± .05	
♂ Chloride deficient	1.24 ± .02	1.36 ± .02		1239	1.61 ± .04	
♀ Control	1.21 ± .04	1.40 ± .04		1418	1.62 ± .08	
♀ Chloride deficient	1.33 ± .04	1.49 ± .03		1218	1.84 ± .07	
Magnesium						
♂ Control	0.050 ± .001	0.055 ± .001	31.8 ± 0.69	59.1	0.059 ± .002	34.6 ± 1.14
♂ Chloride deficient	0.048 ± .001	0.053 ± .001	30.4 ± .76	41.9	0.055 ± .002	33.1 ± 1.42
♀ Control	0.048 ± .0006	0.055 ± .0006	31.8 ± 1.07	52.8	0.060 ± .001	39.7 ± 4.71
♀ Chloride deficient	0.050 ± .0005	0.057 ± .0001	32.9 ± .16	42.0	0.063 ± .001	38.1 ± .56
Phosphorus						
♂ Control	0.636	0.708		773.4	0.768	
♂ Chloride deficient	0.696	0.761		675.0	0.881	
♀ Control	0.589	0.677		675.5	0.758	
♀ Chloride deficient	0.705	0.795		655.6	0.987	

[1] Plus and minus values are the standard errors of the means.

[2] Contents of alimentary tract removed.

Since the individual results were paired variates, the significance of any differences between the control and the chloride deficient rats was determined by Fischer's ('28) modification of "Student's" table. Since little or no difference in mineral composition was found between the sexes, both male and female rats were combined in the comparisons between the chloride and the bicarbonate animals.

By expressing mineral constituents as percentages of the fat-free tissue, the highly variable fat gain was segregated, thereby increasing the statistical significance of the data. Therefore, for the sake of brevity, only the results expressed as per cent of fat-free tissue will be discussed.

There were no significant differences in mineral composition between the male and female rats of the control group, but the chloride deficient animals did show small significant differences between the sexes (as determined by the standard error of the difference). The potassium, calcium, magnesium, and phosphorus constituents of the fat-free bodies were significantly higher in the females than in the males, the differences being respectively, 2.5, 2.5, and 4 times their standard errors.

The chloride content of the chloride deficient rats was 88.9% (value for "P" was less than .01) of that of the control animals. This difference was surprising only in that it was no larger, since the chloride deficient ration contained only 7% as much chloride as did the normal ration. The rat accomplished this adaptation by a slower rate of growth, and by conservation of the available chloride.

When the sodium and potassium composition of the fat-free tissue of the chloride deficient and of the control groups was compared, the composition of the deficient animals in these mineral elements was 95.0 and 96.7% ("P" value less than .01 in both cases), respectively, of that of the controls.

The phosphorus contents of the fat-free bodies of the males and females of the chloride deficient group were 107.5 and 117.4%, respectively, of those of the male and female animals of the normal chloride group. Though statistical tests of significance are lacking for these differences, it would not be fal-

lacious, considering the consistent character of the previous data, to assume that the differences were significant and due to an insufficiency of chloride in the diet.

No real differences were evident between the two groups as to the calcium and magnesium contents of the rat bodies.

The relation of mineral retention to gain of body tissue reveals that the contents of the fat-free tissue gain of the chloride deficient rats in chloride, sodium, and potassium were 72.7, 91.7, and 94.1% ("P" value of less than .01 in all cases), respectively, of the contents of the control animals in these elements.

However, the calcium content of the fat-free gain of the chloride deficient rats was 109.6% ("P" value less than .01) of that of the control animals. This difference signifies that the rats receiving the chloride deficient ration made a greater skeletal growth in proportion to the tissue growth than did the rats receiving the ration of normal chloride content. The phosphorus content of the body gain lends support to this interpretation since the chloride deficient animals gained 122.4% as much phosphorus as did the control rats.

The magnesium content of the gains did not vary significantly between the two groups.

Of the minerals determined, chloride, sodium, potassium, and to some extent, magnesium are of importance as electrolytes; therefore, their relation to body water is significant in relation to the effects of dietary chloride deficiency.

In table 3 the millimole retention of these minerals is related to the kilograms of water gained. Again, the results show a relatively slight variation within the group, and the individual differences are highly consistent.

A comparison of the two groups shows that the concentrations of chloride, sodium, and potassium in the water gain were 78.1, 94.2, and 96.3% ("P" value less than .02 in all cases), respectively, of the concentration of these elements in the water gain of the control animals. The magnesium concentration was not significantly affected by the chloride deficiency.

The chloride deficient rats, to compensate for the lower concentration of chloride, gained a lower percentage of water than did the normal chloride rats (Voris and Thacker, '42), and also reduced the concentration of sodium and potassium in that water.

In addition to the values for mineral retention computed from the body composition, it was planned to determine retention from the analysis of feeds and excreta, but this objective was accomplished only in part, as shown in table 4 in which complete balances are given for sodium, potassium, calcium, magnesium and chloride in the control rats; the balances for the chloride deficient rats being complete with reference to chloride only, and incomplete for the other elements studied.

Though the feces of the deficient rats contained approximately one-half as much chloride as did the feces of the control animals, their chloride retention was 50.3 to 51.3% of that in the feed; whereas, the control rats retained 5.4 to 6.7%. The ability of rats to conserve chloride when their supply is severely limited was illustrated by the urinary excretion of this element. The urinary chloride excretion of the chloride deficient rats was approximately 40% of their intake, or less than 0.6 mg. daily; while the control animals excreted in the urine over 90% of their intake, or nearly 200 mg. daily.

Based on the results of the body analysis of the rats, with both the males and the females the percentage retention of the intake of sodium, potassium, calcium and magnesium was lower for the chloride deficient rats than for the normal controls.

The agreement between the retentions of chloride, potassium, calcium, and magnesium obtained by the two analytically unrelated methods (tables 3 and 4) attests to the accuracy of the analytical work.

The retention of sodium, however, as determined by the two methods, was at variance. The retention calculated from the excreta analysis was, on the average, 38.1 ± 1.52 [2] and 35.9 ± 4.86 [2] % higher for the male and female rats, respec-

[2] Standard error.

TABLE 4

Mineral constituents of the feed, feces, and urine of normal control and of chloride deficient rats. Averages for seven males and five females.

	CHLORIDE	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
♂ Control					
Intake	1504.5	537.9	1710.9	3384	199.1
Feces	21.3	26.0	70.6	1720	51.4
Urine	1385.3	320.8	1362.7	136	87.3
Retention	100.7	191.1	277.7	1528	61.8
♂ Chloride deficient					
Intake	106.3	637.7	1620.9	3316	194.5
Feces	10.5	14.5	38.0	1973	41.6
Urine	42.3				
Retention	53.5				
♀ Control					
Intake	1439.5	514.8	1636.3	3238	190.6
Feces	18.7	25.9	65.1	1625	51.5
Urine	1341.3	315.4	1283.6	156	79.4
Retention	77.3	173.5	287.7	1457	59.7
♀ Chloride deficient					
Intake	101.6	609.6	1549.5	3170	185.9
Feces	10.3	15.7	41.5	1862	39.3
Urine	39.2				
Retention	52.1				

tively, than the values obtained by body analysis. The significance of this difference was not determined.

In harmony with the well-known reciprocal relationship between chloride and bicarbonate ions (Peters and Van Slyke, '31; Greenberg and Cuthbertson, '42) was the finding in the present investigation that the decrease of 8.8 mM in the chloride in the water gain was accompanied by a decrease of 4.4 mM in the concentration of sodium.

Since sodium is present, for the most part, in the extracellular fluids, and potassium is present largely in the intracellular fluids, a decrease in the concentration of sodium would necessitate a similar decrease in the potassium in order to maintain the osmotic equilibrium of the two fluids. The decrease of 4.1 mM in the potassium concentration of the water gain indicated, as did the Na/K ratio, that the diminished sodium concentration was counterbalanced by a similar decrease in the potassium concentration.

SUMMARY

In a 70-day paired feeding experiment comparative data on the metabolism of chloride, sodium, potassium, calcium, magnesium and phosphorus were secured by body analysis of rats on a synthetic diet of normal chloride content (0.28%) and animals on a similar diet in which the chlorides of the salt mixture were replaced by equivalent quantities of corresponding bicarbonates. The chloride deficient ration contained 0.02% chloride and 0.49% bicarbonate. The bicarbonate being considered innocuous, the results obtained were attributed to the deficiency of dietary chloride.

In comparison with the rats receiving the normal chloride ration, the contents of the bodies of the rats receiving the chloride deficient ration were less in chloride, sodium, and potassium and greater in calcium and phosphorus; the contents of the body gain were less in chloride, sodium, and potassium, and greater in calcium and phosphorus; and the concentration of chloride, sodium and potassium was less in the total water and water gain without disturbance of the ratio of

sodium to potassium. The adjustments of the female rats to a restricted intake of chloride were similar to but not as large as those of the male rats.

The percentage retention of the intake of sodium, potassium, calcium and magnesium was lower for the chloride deficient rats than for the normal controls; while the percentage retention of chloride was much higher for the chloride deficient rats than for the controls.

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MEAD JOHNSON AND COMPANY
'B-COMPLEX' AWARD

Nominations are solicited for the 1944 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B Complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calender year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1943 must be in the hands of the Secretary by January 10, 1944. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

ARTHUR H. SMITH
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SECRETARY, AMERICAN INSTITUTE OF NUTRITION